[illegible]YBEAN LEAF GROWTH DURING DROUGHT

By

HAROLD C. RANDALL

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1936

ACKNOWLEDGMENTS

I would like to thank Dr. Thomas Sinclair as chairman of my committee, for supporting my work by providing an assistantship. His patience and wisdom over the years have been invaluable. I would also like to thank the other members of my committee, Dr. Bassett, Dr. Emerson, Dr. Jones and Dr. Boote, for taking the time to discuss the many questions which arose during the course of this work.

Support supplied by Mr. George Drake was essential in turning technical mountains into molehills, and for this I am most grateful. I would also like to thank Dr. Irey from the Mechanical Engineering Department for his assistance with the heat transport study of the psychrometer and Dr. Dan Haile (USDA) for allowing me to use his laboratory for cell counts.

In the final analysis, my family, the Randall's and the Ricca's, held me together throughout the years of work. I would like to express my gratitude to my parents who instilled in me a love of learning at an early age, my children, whose love provided me with the highest purpose, and most of all to my wife. The burdens shouldered by her would have collapsed the strongest of persons. Her love, devotion, financial support, and assistance with field and laboratory work as a technician made possible the completion and the successes of this work.

TABLE OF CONTENTS

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

INDIVIDUAL SOYBEAN LEAF GROWTH
DURING DROUGHT

By

HAROLD C. RANDALL

December 1986

Chairman: Dr. T. R. Sinclair
Cochairman: Dr. G. Smerage
Major Department: Agronomy

Experiments were conducted to characterize the impact of drought on the growth and development of field grown soybean [Glycine max. (L.) Merrill] leaves and to assess the impact of drought on cell division and cell expansion. A hypothesis was proposed which stated that the response of leaves to drought stress is dictated by the development stage of the leaf at the time of drought. Leaf cell populations were more influenced by drought early in the development of a leaf than later. Final leaf areas were also related to the timing of drought. These results suggested that responses to drought were mediated by differential contributions of cell division and expansion during a particular leaf development stage.

An equation which describes the relaxation of individual cell walls was used as a model to characterize the expansion of leaf cells. The turgor potential, leaf wall yield threshold and the leaf relative growth rates were measured in the leaves from field-grown, irrigated

d [illegible] wall extensibility was estimated from these volumes. Leaf turgor potentials were lower in the droughted than in the well-watered leaves. The wall yield threshold was linearly related to the normalized leaf area. Drought increased leaf wall yield thresholds by 0.2 MPa for leaves which had just emerged from the meristem. Leaf relative growth rates were responsive to changes in the level of irrigation and were a function of the normalized leaf area for leaves having completed more than 20% of their growth. The leaf wall extensibility was estimated at between 0.07 and 0.40 $MPa^{-1}\ h^{-1}$ for well-watered and droughted leaves.

A model of leaf growth was constructed which incorporated the wall relaxation equation and a unique functional relationship between cell expansion and division termed the maximum-volume division function. This function requires that cells attain a time-dependent volume before proceeding to a division cycle. The model successfully portrayed leaf growth during well-watered conditions and predicted the nature and extent of drought induced growth reductions. A parameter analysis suggested that only increases in the leaf turgor were capable of increasing leaf area

CHAPTER I
INTRODUCTION

The growth of leaves occupied the spotlight in [redacted] botany for almost 20 years with groundbreaking work by Maltherpe (1956) and more recently by Maksymowych (1973). During that time, the basic cellular processes of division and differentiation were temporally located and described. Since that time, the interest in leaves has had more to do with their function as photosynthetic organs than with their rates of formation or development. With the increased need for more efficient crop production, brought on by dwindling resources and increased demand, there has been renewed emphasis on the growth of leaves. In part this is due to the recognition that the leaf is the primary structure responsible for converting solar energy into energy utilizable by man.

The area of an entire canopy of leaves per unit ground surface area, otherwise known as the leaf area index, has come to occupy a central role in many crop growth and yield predicting models (Monteith, 1977; Curry et al., 1975). More recently, the approach of Sinclair and Specth (1982) to yield formation, use the fact that leaf area index dictates the fraction of solar radiation intercepted by a crop. This in turn is related to the amount of biomass produced by an efficiency of conversion coefficient. Grain yield may then be calculated by multiplying the biomass by the harvest index.

[illegible] yield formation was sufficient for use with well-watered field-grown soybeans (Glycine max L. Merrill). A second approach to yield determination requires the calculation of biomass through seasonal integrations of crop photosynthesis and respiration. Both types of modeling approaches are subject to inaccuracies due in large part to their inability to portray leaf area formation during drought. Neither approach to leaf production has the capacity to realistically treat leaf growth during cyclical periods of plant stress.

Thus, there is an important deficiency in current methods for determining yield formation in crops during drought due to the inability to predict leaf growth. This is a major problem since much of what man produces world-wide is subjected to drought. Boyer (1982) estimated that drought is the single-most pervasive cause of reduced yields worldwide. Predictions of yield must take into account these realities.

Another approach employed to predict leaf growth in a variable environment was limited by the use of species and stage-of-growth specific coefficients to relate turgor potential to leaf blade expansion (Zur and Jones, 1981). An excellent work on the growth of cucumber (Cucumis sativus) leaves by Hsiao et al. (1976) provided insight into the factors controlling cell division. However, they resorted to empirical descriptions of leaf expansion since they did not consider, in a mechanistic sense, the role of cell expansion in the growth of leaves.

For models of leaf growth to be effective, explanatory models are required for any hope of flexibility under a variety of conditions (Loomis et al., 1979). Equally important is a realistic expectation of what any model may accomplish. The model of leaf growth developed in this work considers growth reductions due to small to moderate drought. Growth during severe drought may tax current concepts of growth beyond their usefulness or physiological reality. A simple analogy may be drawn to the spring constant in Hooke's Law. This constant simply defines the relationship between the applied force and displacement. Under extreme conditions, this constant fails to relate the force and the displacement. We must not expect all cause-effect relationships to hold under every imaginable condition.

There are three broad parts to this work:

1) the impact of drought on leaf growth,
2) interpretation of responses to drought by analysis of leaf growth into basic components,
3) and synthesis of these processes into a model.

The Impact of Drought on Leaf Growth

Three unique stages of leaf development may be categorized according to their response to drought: initiation, early growth (leaves less than 20% expanded) and late growth (leaves greater than 20% but less than fully expanded). The latter two stages were separable through differing cellular processes. During early growth stages leaves have a high number of dividing cells while the growth of more mature leaves is probably dominated by cell expansion.

[illegible] to drought of each of these stages provides clues to the effect of drought on the underlying mechanisms of leaf growth

In Chapter II, the effect of drought on leaf emergence is examined. Vandeland et al. (1982) examined this stage of leaf development in detail and determined that soybean leaf emergence, as quantified by the plastochron index was a very sensitive indicator of drought. While this aspect of leaf growth is sensitive to drought, leaf area was shown in this study to be more responsive. Chapter III considers the effects of drought on area development as well as provides data on cell number in both young and more mature leaves. Although Yegappan et al. (1980) investigated the long term effects of drought on leaf area and cell number in greenhouse grown sunflower (Helianthus annuus), no experimental data exist for these changes in field-grown soybean. Results from these two chapters suggest that the impact of drought could be predicted from knowledge of the relative importance of cell division and cell expansion at a given phase of leaf growth.

Reduction of Leaf Growth into Its Components

A reductionist approach was used in Chapter IV to interpret the characteristic responses to drought observed in data from Chapters II and III. Because leaf growth consists of cell division and expansion, the understanding of how drought influences these processes would help decipher how leaves grew during drought. In Chapter IV, an equation describing a biophysical relaxation of cell walls, originally proposed

by Lockhart [illegible] was assumed to be the necessary and sufficient description of cell expansion in a plant tissue:

$$(1/V)dV/dt = \phi(P - Y) \qquad (1.1)$$

Equation (1.1) represents the original form of the cell wall relaxation equation where V is the cell volume, t is time (h), ϕ is the wall extensibility ($MPa^{-1}\ h^{-1}$), P is the cell turgor potential (MPa) and Y is the wall yield threshold (MPa). The term, relaxation, refers to the loosening of chemical bonds holding cell wall materials together; it may be thought of as a weakening of the structural integrity of a substance. Much early emphasis went into determining which of these variables controlled the growth of single celled Nitella (Green et al., 1971). More recently, Cosgrove (1986) measured these variables with a pressure-probe in pea (Pisum sativum) stem cells. While equation (1.1) has served as a guide to investigate leaf growth, there have been few field studies which have evaluated all of its terms simultaneously, and there has been minimal work conducted on the influence of drought on each of the terms. In the only direct examination of eqn. (1.1) under field conditions, Bunce (1977) measured leaf turgor potentials and growth rates in the leaves of well-watered soybeans (Glycine max (L.) Merrill) but specifically did not consider the wall extensibility. Van Volkenburgh and Cleland (1986) also used eqn. (1.1) to assess factors responsible for controlling the growth of bean (Phaseolus vulgaris) leaves grown in a controlled environment. However, they too never evaluated wall extensibility nor investigated the consequences of drought on each term in eqn. (1.1).

Experiments were conducted to evaluate all the [illegible] wall relaxation equation in leaves which were either [illegible] or droughted field-grown plants. Since the equation was originally developed for single cell expansion, modifications were made to apply the equation to a community of cells in a leaf. Three terms in this equation were evaluated; the leaf turgor potential, the wall yield threshold and the leaf relative growth rates, while the wall extensibility was estimated from the other terms.

The last significant step was to develop a similarly mechanistic approach to the division of cells. Unfortunately, the theoretical basis of cell division has not progressed as far as it has for cell expansion. Early studies of leaf growth were mainly concerned with the delineation of cell division and cell expansion processes (Miltherpe, 1956). Considerable effort (Dale, 1970 and 1976) was spent on various empirical explanations to account for cell production with time. Wilson (1966) conducted a study of the influence of light on cell production in leaves of cucumber and Miltherpe (1959) also conducted a study on cucumber to determine the influence of temperature on cell division. Neither of these studies nor any of the other studies on cell division, resulted in concepts which could describe the division of cells in a mechanistic way.

Without resorting to pure empiricism, a functional requirement was used to cope with cell division. This requirement simply states that cells must expand before they divide. This allowed the evaluation of cell division in terms of something which could be

measured. The [illegible] is determining when cells [illegible] divide.

In Chapter VI the equations for cell [illegible]n and division were finally assembled in a simple computer model with input conditions derived from data collected and presented in earlier chapters. This model is unique in several important aspects; first, it is a mechanistic model to which it owes its flexibility, and secondly, the concept that cell division requires an expansion phase is integral to the operation of the model. After verification of the model with simulations for the nominal case of leaf area expansion as well as cell population increases with time, a parameter analysis and simulations of drought on leaf growth and development were performed.

In the last chapter, conclusions are summarized and a brief account is given to the multitude of questions that generally arise following any scientific inquiry.

CHAPTER II
[redacted] OF LEAF EMERGENCE TO DROUGHT

[redacted] a process which may be characterized by the emergence [redacted] from the meristematic region followed by expansion of the leaf blade. Leaves must emerge from the meristem before they can contribute to the photosynthetic capacity of the plant, so that an examination of the rate of leaf emergence is a logical starting point for an investigation of the influence of drought on soybean leaf growth and development. Hofstra et al. (1977) likewise began with a study of leaf emergence which served to identify a consistently recognizable plant development process which could be utilized in general crop growth models. This chapter is concerned with the more specific purpose of quantifying the reductions in the rate of leaf emergence due to drought, as well as identifying which process, leaf initiation or expansion, is more sensitive. Furthermore, since the effect of severe drought results in cessation of most growth processes, short to moderate length cyclical droughts were imposed to study leaf growth processes.

The rate of appearance of leaves from the meristem has been intensively studied for a number of crops including soybean (*Glycine max* (L.) Merrill) (Hesketh et al., 1973; Hofstra et al., 1977; Thomas and Raper, 1976; Vendeland et al., 1982) and wheat (*Triticum aestivum* L. em. Thell) (Baker et al., 1986; Baker and Gallagher, 1983). During optimal growth conditions, the rate of leaf appearance is

approximately [illegible] reported that both soybean and [illegible] periods of drought, winter and spring wheat decreased [illegible] between appearance of leaves (Baker et al., 1986). In addition, the plastochron, a measure of the time between the appearance of successive leaves, increased in droughted field-grown soybeans (Vendmiend et al., 1982). In the latter study, it was concluded that the change in the plastochron index was very sensitive to environmental conditions. Leaf initiation in container grown sunflower (Helianthus annuus L.) also showed a reduction in the leaf appearance rate due to drought stress (Marc and Palmer, 1976; Yegappan et al., 1980).

The objective of this research was to determine the influence of short to moderate length cyclical drought on the rate of leaf appearance and to determine the relative sensitivity of leaf blade expansion and leaf appearance rate to drought. Field-grown soybeans were irrigated every 4 or 8 days or were well-watered (irrigated every 2 days). Leaf areas were measured daily on the top-most 5 leaves. For each irrigation cycle, the plastochron index was a good indicator of whole plant leaf area development. The leaf emergence rate as quantified by the plastochron index was shown to be less sensitive than leaf area expansion to cyclical droughts.

Materials and Methods

Soybean (cv. Biloxi) was used as the test material in this and all subsequent experiments. Biloxi belongs to Maturity Group VIII and provided up to three months of vegetative growth when sown in early

April in Gainesville, FL. This cultivar [illegible] large leaves (to 550 cm^2), which facilitated measurement of leaf lamina and observation of leaf emergence from the meristem.

A field experiment was conducted at the Agronomy Farm located at the University of Florida in Gainesville, Florida. The soil in the test plot was an Arredonda fine sand (hyperthermic, coated, Aquic Quartzipsamment). The plot received a preplant application of 400 kg ha^{-1} of 0-10-20 (N P_2O_5-K_2O) fertilizer. Broadleaf weeds were controlled with a preplant application of 3 L ha^{-1} of Trifluralin (alpha, alpha, alpha trifluoro 2, 6-dinitro N, N-dipropyl-p-toluidine). A preemergent application of 4 L ha^{-1} Alachlor (2 chloro-2', -6'-diethyl N (methoxymethyl) acetanilide) was used to control grasses. Demeton (O,O dimthyl S (and O) (2-ethylthio)ethyl] phosphorothioates) and methomyl (S-methyl-n-((methylcarbamoyl) oxy) thioacetimidate) were applied as required to control velvetbean caterpillar and other insect pests.

On 28 March 1982, an 18x30 m field plot was sown at a rate of 40 seeds per meter in 0.9 m rows with a north-south orientation. This plot was divided into three 18x10 m subplots of differing irrigation treatments. Subplots were irrigated every second, fourth or eighth day. The subplot irrigated every eighth day received 30 mm of water while those which were irrigated every second and fourth day received 20 mm of water per irrigation. Irrigation was provided by a 6 m boom attached to a motorized frame which moved on two tracks placed lengthwise in the center of the entire plot. This system was able to supply a uniform distribution of water over each subplot. Irrigation

treatments began on [illegible] May and measurements [illegible] began on 11 May. On 26 May heavy rains forced an end to the irrigation regimes but leaves were measured once more the following day

Leaf Area

The soybean leaf is trifoliolate consisting of a single terminal and two lateral leaflets. Terminal leaflet lengths and widths were measured with a millimeter scale on the top most five leaves of ten plants in each treatment. Measurements commenced when the 5th or 6th leaf had just emerged from the meristem. After the 4th leaf had ceased growing, the lengths and widths of the terminal leaflets from leaves occupying the first (primary leaf) through fourth position were also measured. To relate terminal leaflet dimensions to individual leaf area, a separate group of leaves was harvested and the lengths and widths of the terminal leaflets were measured along with the areas of the entire blade. Leaf areas were measured with a leaf area meter (Model LI-3000, Lambda Instruments, Lincoln, Nebraska).

Plastochron Index

The plastochron index (PI), is a count of the number of leaves on a plant. Leaves which are longer than a specified length increase the plastochron index by one, whereas leaves which are smaller than the specified length are assigned a fractional amount of growth. The plastochron index was used to quantify the impact of drought on the rate of appearance of leaves from the meristem

The plastochron index (eqn 2.1), as developed by Vandeland et al. (1982), was determined for each treatment throughout the 23 day experimental period.

$$PI = n + \frac{\log (L_{n+1}) - \log (R')}{PR} \qquad (2.1)$$

A reference length of the terminal leaflet which defines the leaflet length at emergence from the meristem is denoted by R'. A reference length of 10 mm was used. The n+1 leaf is the topmost leaf whose terminal leaflet length (L_{n+1}) is greater than or equal to R'. The number of leaves below the n+1 leaf is denoted by n. The plastochron ratio (PR) is defined as the logarithm of the ratio of the lengths of the two topmost leaves.

$$PR = \log (L_{n+1}/L_n) \qquad (2.2)$$

The PR for all treatments was set equal to the daily value for the well-watered treatment (irrigated every 2 days) instead of a seasonal average. This tended to reduce the effect of environmental variability on the decimal portion of the plastochron index which was not associated with the irrigation treatments. This particular formulation of the plastochron index (eqn. 2.1) was employed since it directly compares the growth of the n+1 leaf with an unstressed one. Vandeland et al. (1982) have shown that this formulation of the plastochron index is very responsive to the imposition of drought and to recovery after an irrigation.

Results and Di[illegible]

Leaflet Dimensions and Leaf Area

Correlations between terminal leaflet din[illegible] area were made with to facilitate repeated non destructive measurement of numerous field samples. No statistical differen[illegible] were found for any correlations when droughted leaves were compared with well watered leaves so both sets of data were grouped together. When the products of terminal leaflet length (L, cm) and width (W, cm) were regressed against the entire leaf area (A, cm^2), the following equation resulted;

$$A = 2.03*L*W \quad (r^2=0.96) \qquad (2.3)$$

In a study of 12 soybean cultivars, Wiersma and Bailey (1975) found a similar relationship between leaf area and dimensions of the terminal leaflet.

Plastochron Index

The plastochron indices for treatments irrigated every 2, 4 or 8 days are presented in Table 2.1. Plastochron indices were different on most dates among the three irrigation treatments. Throughout most of the experimental period, plants irrigated every 2nd day had the highest plastochron indices while those irrigated every 4th day had plastochron indices lower or equal to those irrigated every 8 days. A heavy infestation of yellow nutsedge (Cyperus esculentus) in the plot irrigated every 4th day may have been responsible reduced growth. Over the entire experimental period, plastochron indices were

[illegible] soybean [illegible] 1982.

Day of year	Irrigation interval		
	2 days	4 days	8 days
		Pl	
125	6.3a[2]	6.0b	5.8c
127	6.9a	6.5b	6.9a
129	7.4a	6.7b	7.3a
130	7.7a	7.1b	7.5a
131	7.9a	7.3b	7.7c
132	8.3a	7.5b	7.9c
133	9.1a	7.9b	8.1c
134	8.6a	7.9b	8.3c
135	8.9a	8.1b	8.5c
136	9.1a	8.4b	8.9c
137	9.6a	8.7b	9.1c
138	9.8a	8.9b	9.1c
139	10.2a	9.1b	9.5c
140	10.4a	9.6b	9.6b
141	10.9a	9.8b	9.7b
142	11.6a	10.1b	10.6c
144	11.4a	10.9b	10.5c
147	12.6a	11.8b	11.7b

[2]Values within a row followed by the same letter are not significantly different at the (alpha= 05) level

approximately 8 and 6% lo[illegible] and [illegible], irrigation regimes relative to those irrigated every 2nd day, respectively. Marc and Palmer (1976) observed greater reduction (35% by the 10th day) of leaf initiation in sunflower plants subjected to a 10 day drought under controlled environment conditions. Under field conditions, Vendeland (1981) observed approximately 36% reduction in the appearance of leaves as measured by the plastochron index in soybeans droughted for 36 days. Due to the short, periodic nature of droughts used in the present study (8 day maximum), relative to Vendeland's work with field-grown soybean, leaf production rates were not as seriously reduced.

Leaf Area

Average leaf areas per plant are presented in Table 2.2 for plots irrigated every 2, 4 or 8 days. Generally, leaf areas were significantly different between plots irrigated at 4 and 8 day intervals compared to those irrigated every second day. Leaf area development was slightly retarded in plants irrigated every 4 days possibly due to the presence of yellow nutsedge. However, by the final week of the experimental period, plants irrigated every 4 days did achieve higher leaf areas than those irrigated every 8 days.

Plant leaf areas plotted against plastochron indices for each treatment are shown in Fig. 2.1. Linear regression analysis of leaf area against the plastochron index resulted in the following equations for treatments irrigated every 2 days (eqn. 2.4), 4 days (eqn. 2.5) or 8 days (eqn. 2.6).

= 769*PI - 1282 (r^2=0.98)

= 208*PI - 908 (r^2=0.97)

= 709*PI - 854 (r^2=0.98)

where A is the whole plant leaf area (cm^2) and PI is plastochron index (eqn 2.1). The relationship between number of leaves produced and plant leaf area is excellent for both well-watered plants (2-day interval) and those irrigated every 4 or 8 days. Similarly, Sinclair (1984) calculated three coefficients based on an allometric relationship between the plastochron index and whole plant leaf area of well-watered, field-grown soybeans and found a linear relationship between leaf area and plastochron index after the plant had 6 leaves.

Relative Sensitivity of Leaf Emergence and Leaf Area Expansion to Drought

Relative to plants irrigated every 2 days, leaf areas averaged over the entire experimental period were reduced approximately 25 and 22% in plants irrigated every 4 and 8 days, respectively (Table 2.2). In contrast to these reductions in leaf area, the plastochron indices of plants irrigated at 4 and 8 day intervals were approximately 7% lower than those measured in plants irrigated every 2 days. Muchow et al. (1986) also found greater sensitivity to drought in leaf area development than in leaf production for field-grown soybeans. Data from Tokasi et al. (1981) may also be used to calculate a greater sensitivity of leaf area expansion than leaf emergence in 4 greenhouse-grown sunflower cultivars. The nature of the protection to drought afforded leaves while they are being initiated has yet to be

Table 2 - Leaf area per plant of field-grown so[illegible] irrigated every 2, 4 or 8 days, 1982.

Day of year	Irrigation interval		
	2 days	4 days	8 days
	Leaf area (cm^2 plant^{-1})		
125	500a[a]	504a	557b
127	609a	526b	600a
129	726a	566b	637c
130	779a	596b	669c
131	823a	624b	688c
132	859a	667b	730c
133			788
134	1002a	756b	819b
135	1151a	829b	853c
136	1153a	862b	944c
137	1208a	908b	990c
138	1251a	958b	1022c
139	1394a	1062b	1050b
140	1504a	1101b	1076b
141	1553a	1174b	1123b
142	1720a	1286b	1226b
144	1880a	1427b	1352b
147	2274a	1693b	1622b

[a]Values within a row followed by the same letter are not significantly different at the (alpha=.05) level.

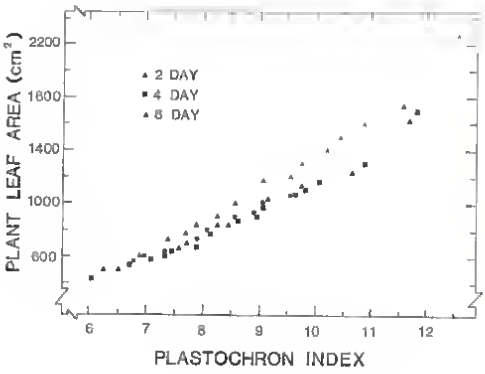

Figure 2 1 Whole plant leaf area plotted as a function of the plastochron index for plants irrigated at 2, 4, or 8 day intervals, 1982.

[illegible]solved. Barlow et al. (1977) has suggested that osmotic [illegible] in the apex of droughted wheat contributes to survival and r[illegible] following drought

Conclusions

During optimal growth conditions, the plastochron index is a simple function of the average daily temperature above a base temperature. Sinclair (1984) showed that for well-watered plants, a simple allometric relationship exists between the plastochron index and the leaf area. This allowed a straight forward approach to obtain the whole plant leaf area based only upon the average daily temperature and three cultivar-specific growth coefficients. Results suggest that a linear relationship exists between the plastochron index and leaf area for plants which had been subjected to short to moderate length cyclical droughts. The leaf emergence rate, as described by the plastochron index, was less sensitive to drought than was leaf area expansion.

CHAPTER III

[illegible] DEVELOPMENT [illegible]

In light of the greater sensitivity to drought of leaf area expansion than leaf emergence and the need to develop a coherent basis of drought's impact on leaf growth and development, studies were undertaken to explore the mechanisms of drought-induced reductions in leaf area.

To date, the behavior of area development of individual leaves has not been truly predictable. Drought may effect cell division and/or expansion but there seems to be little consensus on the mechanism by which drought exerts its influence. Terry et al. (1971) observed no reduction in cell expansion but measured a reduction in the rate of cell division in leaves of droughted sugar beet (Beta vulgaris L.). Clough and Milthorpe (1975) concluded that leaf cell division was less sensitive to drought than was leaf cell expansion in a tobacco hybrid (Nicotiana tabacum x N. tomentosa). McCree and Davis (1974) inferred from epidermal cell counts of sorghum (Sorghum bicolor L. Moench) that cell division and cell expansion both played a part in the response of leaf growth to drought. To add to the confusion of drought's impact, some workers reported an increase in palisade cell volume with drought (Berlin et al., 1982), others found reductions in cell volume during drought (Bletyet, 1967) and still others determined that no reduction in cell volume occurs in response to drought (Terry et al., 1971).

Clearly there has been little success in establishing a coherent framework from which to build a unified theory of leaf growth response to drought. As a first step towards building such a framework, a basic principle or hypothesis of leaf growth can be stated as follows: response to drought is in large part dictated by the developmental stage of the leaf at the time of drought. A corollary to this hypothesis is that the underlying reason for the various responses observed at different organ ages is due to the differential sensitivities between cell division and cell expansion. Young leaves have been shown to have a higher percentage of dividing cells than more mature leaves (Milthorpe, 1959; Sunderland, 1960). Depending on the relative sensitivities of cell division and expansion to drought, there might be a differential sensitivity of small versus larger leaves to drought. To test these and other consequences of the hypothesis, experiments were performed over a three-year period on field-grown soybeans. An attempt to discover a drought sensitive stage or leaf age was an important test of the hypothesis. Drought sensitivity was investigated by measuring the cell number and leaf area in fully expanded irrigated and droughted leaves when drought was initiated at different stages of leaf development.

The results confirmed that leaf developmental stage is a critical factor in determining the drought response and, in particular, the most sensitive leaf stage in soybean corresponds to the period between emergence from the meristem until soon after the leaf has unfolded (20% of final leaf area for the cultivar examined). The data further suggest that the differential responses of various leaf ages to

drought was due to the [illegible] importance of cell division and expansion associated with a particular stage of growth.

Materials and Methods

A series of field experiments beginning in 1982 and spanning three years were conducted to evaluate the gross morphological and cellular changes in soybean leaves associated with drought. All experiments were conducted at the Agronomy Farm located at the University of Florida in Gainesville, Florida. Cultural practices for all experiments were similar to those described in Chapter II.

1982

On 2d March an 16x30 m field plot was sown at a rate of 40 seeds per meter in 0.9 m rows in a north-south orientation. This plot was divided into three 16x10 m subplots of differing irrigation treatments. Each subplot was irrigated every second, fourth or eighth day. The subplot irrigated every eighth day received 30 mm of water while those which were irrigated every second and fourth day received 20 mm of water per irrigation. Irrigation was provided by a 6 m boom attached to a motorized frame which moved on two tracks placed lengthwise in the center of the plot. This system was able to supply a uniform distribution of water over each subplot. Two 9-day cycles were studied, the first cycle being initiated on 3 May when the 6th leaf had emerged on most plants and the second cycle on 12 May. Terminal leaflet lengths and widths were measured on the top most five leaves of ten plants in each of the three treatments. The 6th through

9th fully expanded leaf [illegible] number determinations. Three plants were sampled in each of the three irrigation treatments. Leaves were placed into formalin acetic acid alcohol fixative (FAA) (Johansen, 1940) immediately following harvest and were transferred to 75% alcohol within a month.

1983

A second experiment was conducted to follow changes in cell number during development of droughted and irrigated leaves and to identify more precisely when leaf cell numbers were reduced by drought. Due to delays in obtaining the use of a rain shelter, only well-watered leaves were sampled during their development. The field conditions were essentially the same as the 1982 experiment. Cell numbers of soybean trifoliolate leaves from the 6th and 7th node were sampled throughout their development on well watered plants. Due to poor preservation of the tissue from small leaves (those with areas less than 30 cm^2) in 1982, small leaves were placed in Navashin's fixative (Johansen, 1940) and the larger leaves were placed into a 75% solution of formalin acetic acid-alcohol fixative (FAA).

1984

An experiment to determine the effect of a one-time, eight-day drought stress on final leaf dimensions and cell number was conducted on the 6th through the 15th leaf. Terminal leaflet lengths were measured at the start of the eight-day drought and again after the 15th leaf was fully expanded. The mean leaf areas at the time of

[illegible] cm [illegible] /th leaf -- 92 cm^2, 8[illegible] 1 3 [illegible] and 9th leaf 7 cm^2 Samples for cell numbers we obtained from fully expanded leaves and treated as before (1983).

Throughout May and June a second experiment examined the relative growth rates of well-watered leaves over a 12 h interval Terminal leaflet lengths were measured with a millimeter scale but those less than 25 mm long were measured with calipers Leaf areas were also expressed in terms of a normalized leaf area This was calculated by dividing the area of the leaf at the time of the first measurement by the plot averaged final leaf area at a specific node

Leaf Area

In 1982, to relate leaflet dimensions to total individual leaf area, the lengths and widths of the terminal leaflets were measured along with the areas of the entire blade. Leaf areas were measured with a leaf area meter The best fit was established between leaf area and terminal leaflet length and width with a linear regression analysis (see Chapter II).

To increase the speed and number of leaf area samples obtained, a relationship was established between the length of the terminal leaflet solely and the area of the entire leaf In 1983, terminal leaflet lengths and leaf areas were measured on approximately 500 leaves Regression analysis was performed on the relationship between terminal leaflet length and whole leaf area.

Cell Number

To prepare leaves for [illegible] washed in ethanol for 5 minutes. The total area of [illegible] leaflet was measured with a leaf area meter followed by removal of the terminal leaflet for subsequent cell number analyses. The midrib from the terminal leaflets were dissected from the leaf blade, and the leaf blade was cut with a scalpel into 0.5 cm^2 pieces. The midrib was cut into 5 mm strips. To the midrib and leaf blade were then added 25 ml of 0.30 M sorbitol and 7-15 ml pectinase (Sigma Chemicals). This mixture was placed in 50 ml flasks with 3 stainless steel ball bearings and nuts to provide a gentle abrading of the tissue. The flasks were then shaken for 24 h at a rocking rate of 180 min^{-1} in a temperature controlled water bath set to 37°C. Cells which had been separated from the bulk of tissue were decanted off and stored at 5°C until all cells had been freed. When all of the tissue was completely macerated, cells were suspended and samples taken for counting in a hemocytometer.

Results and Discussion

Leaflet Dimensions and Leaf Area

Correlations between terminal leaflet dimensions and whole leaf area were made to facilitate repeated non-destructive measurement of numerous field samples. In 1982, equation (2.3) was used to calculate leaf area data from the length and breadth of the terminal leaflet. Leaf area data from 1983 and 1984 were derived from a relationship

established between the length of the terminal leaflet (cm) and the total leaf area (cm^2) which are plotted in Fig. 3.1. Individual leaf area was estimated by the following regression equation:

$$A = -0.28 + 1.259*L^2 \qquad (r^2=0.995) \qquad (3.1)$$

Wiersma and Bailey (1975) found a similar relationship between leaf area and the length of the terminal leaflet squared. The use of either terminal leaflet lengths only or lengths and widths proved to be an accurate method to follow changes in the leaf area of field grown soybeans.

Sensitivity of Leaf Area Development to Drought

Leaf areas for the 6th, 7th, 8th and 9th leaves are plotted against day of the year for the three irrigation treatments of 1982 in Fig. 3.2 through Fig. 3.5. The growth of drought stressed young leaves was retarded to a greater extent than the growth of larger leaves. By day 130, the 8th leaf had emerged in the treatments irrigated every 2nd and 8th day. After day 130, growth of the 8th leaf in the latter treatment lagged four to five days behind the growth of the 8th leaf in the former treatment. If one considers the point in time when the 8th leaf from plants irrigated every 8 days was originally observed to have emerged from the canister (day 125), then growth of that leaf lagged 10 days behind the growth of the 8th leaf from plants irrigated every 2 days. In contrast to the lag in growth experienced by the small droughted leaf, the growth of the 7th leaf from plants irrigated every 8 days (which had areas on day 129 that were essentially indistinguishable from plants irrigated every 2 days)

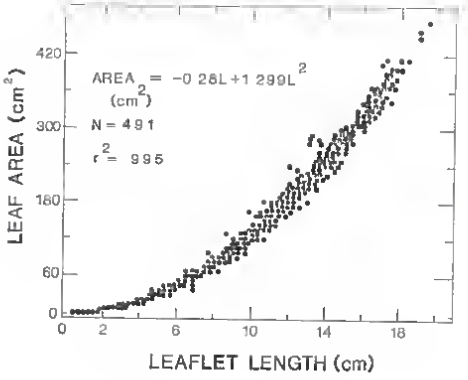

Figure 3 1 Soybean leaf area per leaf as a function of the length of the terminal leaflet

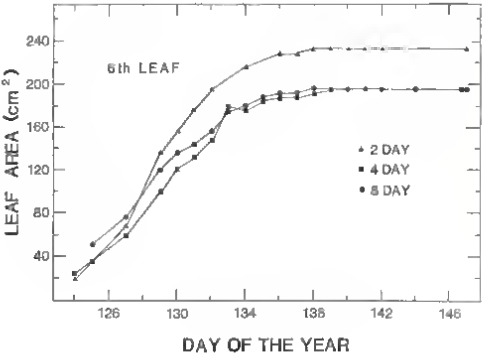

Figure 3.2 Development of the 6th leaf on plants irrigated either every 2, 4 or 8 days over two experimental periods in 1982

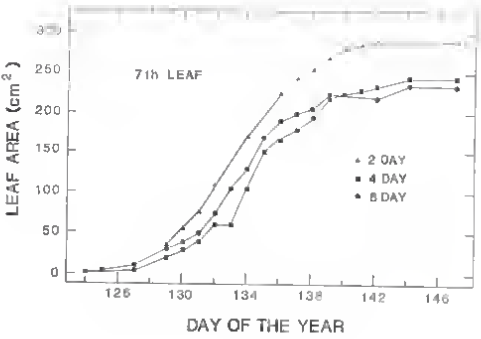

Figure 3.3 Development of the 7th leaf on plants irrigated either every 2, 4 or 8 days over two experimental periods in 1982

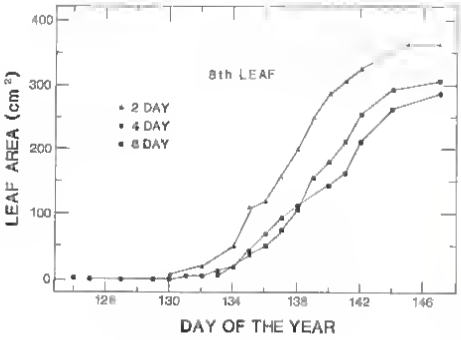

Figure 3.4 Development of the 8th leaf on plants irrigated either every 2, 4 or 8 days over two experimental periods in 1982

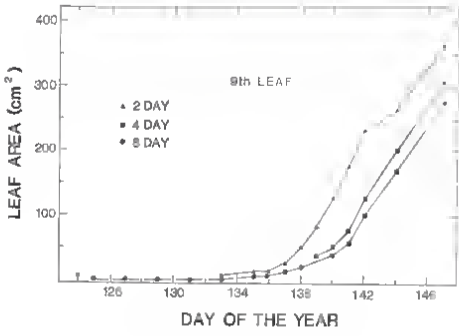

Figure 3 5 Development of the 9th leaf on plants irrigated either every 2, 4 or 8 days over two experimental periods in 1982.

[illegible] of the 7th leaf from plants irrigated every 2 days [illegible]

As a measure of the sensitivity of small leaves to drought stress, Vendeland et al. (1982) proposed use of the plastochron ratio (eqn. 2.2). It is the ratio of the length of the youngest leaf to the length of the next oldest leaf. If drought reduces the expansive growth of the small leaf more than that of the older one, then the ratio becomes more negative. On day 130 the PR (leaf 8/leaf 7) was 0.47±0.02 and -0.57±0.03 (mean ± standard error) for the treatment irrigated every 2nd and 8th day, respectively. The sensitivity of small leaves to drought was also noted in the work of Vendeland et al. (1982) with soybeans. Furthermore, they proposed that the greater growth reductions due to drought observed in small than in larger leaves may have been due to the greater sensitivity of cell division than cell expansion.

Calculations of relative growth rates further illustrates that small leaves, and in particular, leaves which are still folded or in the process of unfolding, display growth characteristics distinctly different from larger and more mature leaves. These differences may play a role in the differential sensitivities of small and large leaves to drought stress. Plots of leaf relative growth rates as a function of normalized leaf area for plants irrigated every 2nd day (1982) are presented in Fig. 3.6. There is a clear division of leaves into two groups separated by the vertical line at 0.2 normalized leaf area units. Leaves which are still in the process of unfolding lie to the left while those which have unfolded are located to the right of

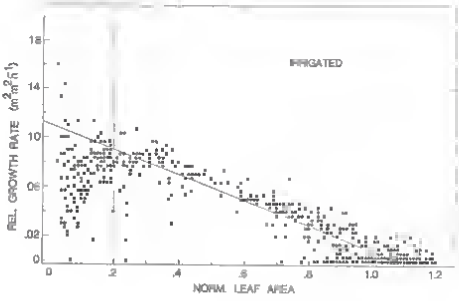

Figure 3.6 The relative growth rates of leaves from plants irrigated every 2nd day are plotted against their normalized leaf areas

[illegible] lines. Small leaves have both high and low growth rates but a majority have low relative growth rates. Larger leaves tend to have relative growth rates along a descending line from the highest values to a point where leaves have attained full expansion (1.0 on the ordinate). It appears that once leaves have unfolded, their growth may be so dominated by cell expansion processes that the influence of cell division on the overall leaf growth rate is masked. If true, then drought prior to leaflet unfolding may play a significant role in cell population dynamics.

A second major influence of drought on growth and development of soybean leaves is the effect on the final leaf area. Reductions in final leaf area of leaves irrigated every 4 and 8 days is clearly smaller (Figs. 3.2 to 3.5). Final leaf areas of the 6th through 9th leaf and for treatments irrigated every 2nd, 4th and 8th day are presented in Table 3.1. When compared with areas of leaves from plants irrigated every 2nd day, final leaf areas were reduced approximately 15% for plants irrigated every 4 days and from 15 to 24% for plants irrigated every 8 days. These reductions were caused by multiple, short duration droughts throughout the growth of the 6th through 9th leaf. Therefore, under these conditions it was not possible to ascribe a particularly sensitive stage to the effects of drought or separate the effects on cell division versus expansion.

[illegible] 6th and 9th leaf from [illegible] 2, 4 or 8 days in 1982

Irrigation interval (Days)	6	7	8	9
		Area (cm^2)		
2	234	290	360	360
4	196	243	309	303
8	198	236	290	275
L S D $_{0.05}$	15	20	23	21
		Cells*10^7		
2	53.5	58.5	61.6	64.1
4	41.2	52.7	54.8	51.0
8	42.8	49.7	51.1	41.7
L S D $_{0.05}$	3.8	2.9	3.4	4.4

Sensitivity of Cell Number and Final Leaf Area to Drought

To fully assess the influence of drought on the growth of soybean leaves, leaf cell populations as well as leaf area must be evaluated. Leaf cell numbers were counted during the development of well-watered soybean leaves in 1984 to provide background on the timing and rate of cell division. Leaf cell number and corresponding leaf area for a well watered 6th and 7th leaf are presented in Fig. 3.7. Cell number seemed to increase initially in an exponential manner as other studies have shown (Dale, 1976; Malthorpe, 1956; Makeymowych, 1973), but in the present study the infrequency of samples during early leaf growth made it difficult to determine the coefficients of exponential growth with any certainty. However, these data indicate that the exponential increase in cell number may have occurred only during the first 7 to 8 days of growth. At that time, the area of the 6th leaf is approximately 50 cm^2 and it has been unfolded for 1 day. Thereafter, cell numbers increased roughly linearly until the 14th day for the 6th leaf and until full expansion for the 7th leaf on the 22nd day. Sunderland (1960) found that an exponential increase in the number of lupin (Lupinus albus) cells continued for only five days after leaf emergence. Wilson (1966) also found an Cucumber (Cucumis sativus) leaf cells increased exponentially for only four days beyond leaflet unfolding. Leaf area at that time was only a fraction of the final fully-expanded leaf area. In bean leaves (Phaseolus vulgaris), Dale (1964 and 1970) found a rate of increase in cell number similar to that in Fig. 3.7. Consequently, the literature supports the

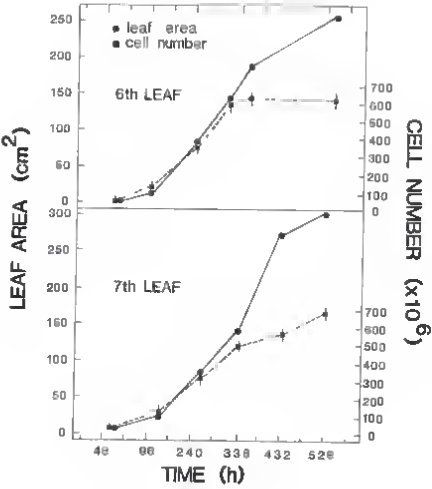

Figure 3-7 The leaf area and the number of cells during the growth of the 6th and 7th leaves from well watered plants in 1984.

[illegible] decrease in cell numbers occurs [illegible] plant species only during early leaf growth.

As presented earlier, drought caused a reduction in the final [illegible] that a leaf attained as well as caused a reduction in the growth [illegible] of small leaves relative to the growth of larger leaves. This suggests that drought causes a reduction in the cell division rate. Final leaf areas may be reduced by drought because if fewer cells are produced than the total volume these cells occupy will be proportionately smaller. As a partial test of these statements, in 1982, leaf cell populations were counted in well-watered (irrigated every 2nd day) and droughted (irrigated every 4 or 8 days), fully-expanded leaves. Leaf cell populations from fully expanded leaves are presented in Table 3.1 for the three irrigation treatments in 1982 (2, 4 or 8 day irrigation cycle). Relative to the 2-day irrigation regime, cell numbers were reduced 23, 10, 11 and 19% for plants irrigated every 4 days and 20, 15, 17 and 24% for the 6th through 9th leaves of plants irrigated every 8 days. Because leaves were droughted throughout their growth, there was no pattern in the reduction of cell numbers with leaf position. To help assess the importance of final cell number (FCN) in determining the final leaf area (FLA), linear regression analysis was performed which resulted in the following relationship:

$$FLA = 5.93E10^{-7} * FCN - 33.7 \quad (r^2 = .80) \qquad (3.2)$$

Reductions in cell number roughly correspond to reductions observed in the areas for the leaf presented in Table 3-1, so drought may reduce leaf area in part by reducing the number of cells produced.

[illegible] the final number of cells and leaf [illegible] at the 6th through 15th nodes of [illegible] droughted for 8 days, relative to leaves from well watered plants, 1984

Leaf position	Area on 1st day without water (cm^2)	Cell number % change		Leaf area % change	
6	154	0	NS[z]	1.5	NS
7	92	0	NS	+0.2	NS
8	20	0	NS	-3.0	NS
9	7	4.3	*	-11.2	*
10	2	-16.0	*	10.0	*
11	1	-10.0	*	-8.0	*
12	<1	-6.5	*	-4.0	*
13	<1	+2.5	NS	-3.0	NS
14	<1	+3.0	*	-3.0	*
15	<1	+2.4	NS	+0.5	NS

[z]Well-watered and droughted final leaf area means are significantly different at the (alpha=.05) level of significance (*) or are not significant (NS)

In 1984, [illegible] of a single moderate drought (8 days) on the final area and cell number of the 6th through the 15th leaf was measured and is presented in Table 3.2. The greatest relative reduction in final leaf area resulted when the leaves had recently emerged from the meristem at the time of the drought (9th node, 7 cm^2). The crop may have been able to rely on water stored in the soil profile so that the 9th leaf may have been slightly larger by the time the stress was perceived. Despite the ambiguity about how soon the effect of drought influenced leaf growth, it seems as though leaves less than 10% fully expanded (based upon a final area of 300 cm^2) were the most influenced by drought. Similarly, Yegappan et al. (1982) droughted sunflower and observed that the greatest magnitude of final leaf area reduction occurred when the stress was imposed at the time of unfolding.

To determine the effect of drought on leaf cell division, cells were counted in fully-expanded leaves in the 1984 experiment. Sensitivity of any particular stage of leaf development to drought would be indicated by the relative reduction in the number of cells. The percent changes in the number of leaf cells in the fully-expanded leaves from droughted plants relative to those from well-watered plants are presented in Table 3.2 along with the area of the leaf at the time drought was initiated. The greatest reduction in the number of cells due to drought occurred in the smallest visible leaf to have emerged from the meristem (11th node) at the time the drought was initiated. Reductions in the number of cells produced were insignificant for leaves which were still located within the

drought.

In contrast to previous drought cycles during leaf development (Table 3.1), changes in the area of individual leaves did not correspond in magnitude or timing to the changes in cell number (Table 3.2). That is, a 40% reduction in cell number did not necessarily result in a similar reduction in the leaf area. The greatest reduction in leaf area occurred for larger leaves at the initiation of the drought than it did for leaf cell number. This is consistent with evidence in Fig. 3.2 that cell division ceases in leaves which have nearly completed their growth, so that drought would have minimal effect on cell division rates in larger leaves. However, drought may still influence the expansion of cells not fully expanded. This result shows the affect of drought on young leaves and support the hypothesis that drought alters soybean leaf growth through its impact on both cell division and expansion. This also indicates that the developmental stage of the leaf at the time of initiation of drought is crucial to the magnitude of the effect of drought on final cell numbers and leaf area.

Conclusion

This research was designed to examine the hypothesis that changes in leaf area due to drought are dictated by the leaf developmental stage when drought is first perceived. This was established during 1984 when the effects of a single moderate drought reduced the greatest proportion of leaf area for leaves still in the process of

unfolding. As a consequence of this hypothesis, it was inferred that the responses to drought are mediated by differential contributions of cell division and cell expansion during a particular leaf developmental stage. As presented in Table 3.2, the greatest reductions in area occurred in leaves which were larger than those younger leaves which showed similar reductions in cell number. Further support of the hypothesis came from the heightened sensitivity of small leaves to drought indicated in Table 3.2. Leaves droughted late in their development showed minor reductions in leaf cell numbers and leaf area while leaves droughted earlier in their development displayed considerable reductions in final cell number and leaf area

CHAPTER IV
[illegible] AND WALL YIELD THRESHOLD OF [illegible]OWN SOYBEANS DURING DROUGHT

The irreversible increase in the size of plant cells (termed expansive growth) is characterized by both a flux of water into the cell and by an irreversible extension of their walls. A biophysical description of cell growth is thought to first require a weakening of the structural integrity of the cell wall to the point where the wall begins to extend irreversibly. This point is termed the cell wall yield threshold. As a consequence of this extension, the internal pressure of the cell contents against the cell wall declines. This in turn creates a thermodynamically favorable gradient for water flow from the exterior of the cell to the interior. The flux of water into the plant cell then increases the volume of the cell. Equations have been developed (Lockhart, 1965; Green et al ,1971) which simply express these changes in the cell wall (eqn 4.1) and the flux of water into the cell (eqn 4.2) during expansive growth:

$$(1/V)dV/dt = \phi(P-Y) \qquad (4.1)$$

$$(1/V)dV/dt = L(\sigma \Delta w \quad P) \qquad (4.2)$$

Table 4.1 lists the symbols used in these equations. Equation 4.1 indicates that the relative change in cell volume due to wall extension is equal to the turgor in excess of the cell wall yield threshold times a proportionality factor termed the cell wall extensibility. Equation 4.2 expresses the relationship between the

Table 4.1 Symbols and unit [illegible] and in Chapter IV.

Symbol	Name	Units
	Wall extensibility	$MPa^{-1}\ h^{-1}$
1/V(dV/dt) or RGR	Relative growth rate or rate of water influx	$cm^3\ cm^{-3}\ h^{-1}$
P	Turger	MPa
Y	Wall yield threshold	MPa
π	Osmotic potential	MPa
σ	Reflection coefficient	unitless
L	Hydraulic conductance	$cm^3\ MPa^{-1}\ h^{-1}$

relative change in cell volume due to water entry into the cell is the water potential difference between the inside and the outside of the cell. This water potential difference is equal to the difference between the osmotic potential outside minus the sum of the osmotic potential plus turgor inside the cell. An additional term, the reflection coefficient, is multiplied by the osmotic gradient to account for the permeability of the cell membrane to some solutes. A hydraulic conductance, L, relates the total water potential gradient to the rate of water entry.

An important question is whether the wall relaxation equation (eqn. 4.1) and/or the water influx equation (eqn. 4.2) may be used as a basis for describing organ growth, or whether some other expression(s) must be utilized. Attempts have been made to combine cell growth equations with water transport properties of tissues to describe organ growth (Cosgrove, 1981; Molz and Boyer, 1978; Silk and Wagner, 1980) but generally the complexity of the system has overwhelmed all but the simplest of cases. However, water transport characteristics of the tissue need not be considered if the tissue water flux is sufficient to meet the needs of expanding cells. Expanding cells have the capacity for short term stored growth which allows them to make up for lost growth once conditions for growth become favorable (Green et al., 1977). Short term water deficits due to low tissue water transport conductance may therefore be inconsequential provided that at the very least, leaf cell water potentials come to equilibrium with xylem water potentials during the night.

Cell water conductance also appears [illegible] tissues for several reasons. First, water tr[illegible] reversible while the changes associated with wall r[illegible] irreversible; therefore, growth is not restricted by cell water transport difficulties but by changes associated with the cell wall (Cosgrove, 1986). Secondly, calculations of hydraulic conductance for pea cortical cells have been shown to be sufficiently high to maintain rapid and sustained growth rates (Cosgrove and Cleland, 1983; Cosgrove, 1985).

Since either tissue nor cellular water transport properties appear to be of consequence when considering net diurnal changes in leaf cell volumes, and because irreversible growth is only associated with irreversible changes in the cell walls, it is necessary then to only evaluate the cell wall relaxation equation (eqn. 4.1) for cells in the leaf to obtain leaf cell growth rates. In order to create consistent usage with other water relations terminology, the cell wall relaxation equation was modified from one which applies to cellular water relations to one which applies to bulk properties of all cells in a leaf. To distinguish the cell wall relaxation equation from the modified form for leaves, the word "cell" was dropped and "leaf" was added to each term in the equation (cell wall yield threshold was renamed the leaf wall yield threshold).

In this chapter, two parameters of the wall relaxation equation, the turgor potential and wall yield threshold, will be examined. The objective was to determine values for these parameters under field conditions. Equally important was the evaluation of the influence of

drought as the behavior of turgor maintenance and wall yield thresholds in field-grown soybeans (Glycine max (L.) Merrill) leaves.

The wall yield threshold has not been examined in any plant tissue or organ under droughted, field-grown conditions. Yet, it is claimed that the effective turgor, the difference between turgor potential and the wall yield threshold, is the principal regulator of growth in many studies (Green, 1968; Green et al., 1971; Van Volkenburgh and Cleland, 1986). Turgor potentials and growth rates were measured by Bunce (1977) in well watered soybean leaves grown in in growth cabinets, greenhouses and under field conditions. One can calculate from his data that wall yield thresholds ranged from 0.02 to 0.15 MPa. With bean (Phaseolus vulgaris L.) seedlings raised in growth cabinets, Van Volkenburgh and Cleland (1981) inferred from turgor and growth rate data that the wall yield threshold was approximately 0.45 MPa for leaves placed under white or red lights. Using a similar technique, primary leaves of bean seedlings grown in liquid culture (Davies and Van Volkenburgh, 1983) were found to have wall yield thresholds less than 0.20 MPa. When the wall yield threshold of growth chamber-grown bean leaves was measured with vapor pressure psychrometery, wall yield thresholds averaged 0.24 MPa (Van Volkenburgh and Cleland, 1986). Using micro-pressure probe and psychrometer techniques on dark grown pea stems (Pisum sativum L.), Cosgrove et al. (1984) measured wall yield thresholds of approximately 0.28 MPa. These laboratory studies of the wall yield threshold were needed to expand the repertoire of measurement techniques and to establish the theoretical groundwork of the biophysical control of

[illegible] How [illegible] [illegible] are essential to determine the magnitude of the wall yield threshold needed for predictions of [illegible] and crop growth

To evaluate the wall relaxation equation in leaves of irrigated and droughted field grown soybeans, the leaf wall yield threshold or leaf turgor were measured diurnally as well as seasonally over three years of experimentation. It was found that turgor of mature leaves follows a typical sine wave-like change diurnally. Maximum leaf turgors from droughted plants were lower than those measured from leaves of well-watered plants. Wall yield thresholds were determined with vapor pressure psychrometry at night or early morning prior to sunrise. Wall yield thresholds of small leaves, which had recently emerged from the meristem, were higher in droughted leaves than in well-watered ones and differed in magnitude by approximately 0.20 MPa. Leaf wall yield thresholds increased as the leaf approached full expansion

Materials and Methods

Turgor Potential Measurement

Leaf tissue excised from soybean leaves was placed in brass psychrometer chambers and allowed to equilibrate in a 30°C water bath for 3.5 h before reading total water potentials with a dew-point microvoltmeter. After total water potentials were recorded, the chambers with leaf tissue samples contained within were removed from the psychrometer and sealed with a screw-on cap which had been coated with petrolatum jelly. These units were then placed into a freezer

for [illegible] and 7 days to [illegible] the cell membranes to rupture. To measure the osmotic potential, chambers containing frozen leaf tissue were removed from the freezer, allowed to equilibrate with ambient air (25±3°C) and reattached to psychrometer assemblies within a box containing humified air. The psychrometer assemblies containing the leaf tissue were then placed in a water bath at 30°C for 4 h. Osmotic potentials were read and recorded with a dew point microvoltmeter and strip chart recorder, respectively. Total and osmotic water potential data were converted from microvolts to units of pressure (MPa) by means of a standard equation. This equation was obtained by measuring psychrometer voltage output when equilibrated over KCl solutions of known water potential. Leaf turgor was calculated as the difference between the osmotic and total water potentials.

Measurement of the Wall Yield Threshold in Vapor Pressure Psychrometers

Using vapor pressure psychrometers to measure wall yield threshold is a new method proposed by Cosgrove (1981). He stated that if a piece of growing plant tissue is removed from a source of water, an occurs when a leaf sample is obtained for water potential determination in a vapor pressure psychrometer, the cell walls will undergo relaxation. The relaxation of a cell wall may be imagined as slippage of the bonds holding the cellulose microfibrils of the cell wall together (Cleland, 1976). As the wall loosens, turgor pressure that the cell contents place against the cell wall begins to drop. The final turgor pressure after complete wall relaxation is termed the wall yield threshold. As discussed earlier, the wall yield threshold

minimum turgor which must be exceeded before growth can occur. Wall relaxation only occurs in growing cells, so turgor in mature, nongrowing cells does not change if they are removed from a source of water.

Three conditions must be met before the wall yield threshold may be measured by vapor pressure psychrometery. A first condition is that the turgor potential must be greater than the wall yield threshold at the time leaf tissue is removed from a source of water. This condition may or may not be met in leaves from field grown plants since there may be a significant portion of the day when the turgor potential is below the wall yield threshold. To ensure that the turgor potential is above the wall yield threshold in leaves of field-grown plants, leaves should be sampled during the night when their turgor potential rises due to better water balance. A second condition is that since the wall yield threshold equals the difference of total and osmotic potentials subsequent to wall relaxation, the osmotic potential must remain constant during equilibration. A third condition is that thermal and water vapor conditions within the psychrometer chamber equilibrate prior to measurement of the wall yield threshold. The latter two conditions are discussed in more detail in Appendix A, but briefly, it was found by experimentation and on theoretical grounds that the osmotic potential is essentially constant during the measurement of wall yield threshold in a psychrometer chamber. The heat transport characteristics of a psychrometer chamber assembly, as measured and calculated in relation to the chamber geometry and materials, was found to be sufficiently

rapid. The time required for vapor in a psychrometer chamber as measured and calculated was found to be sufficiently rapid to allow measurement of the wall yield threshold within 2.5 h after thermal equilibration had been completed. Since most tissues require at least 3 h to approach their wall yield thresholds (Cosgrove, 1985), vapor pressure psychrometers provide a convenient means by which to measure this important cell growth parameter. Cosgrove et al. (1984) successfully used this technique to measure the wall yield threshold in pea stems.

Field Experiments

A series of field experiments were conducted over a 3-year period to evaluate leaf turgor and the wall yield threshold of field-grown, irrigated and droughted soybeans. The cultural practices employed in each of the experiments were discussed in Chapters II and III.

1982. An experiment was conducted to evaluate the wall yield threshold over several diurnal cycles. The experimental treatments and plot layout were discussed in Chapter II; however, several additional considerations need to be stated. The treatments consisted of irrigations every 2nd, 4th or 6th day. Two 6-day cycles were imposed, the first being initiated on 2 May when the 6th leaf had just emerged from most plants and the second on 12 May. Total and osmotic leaf water potentials were measured every 4 h during the last day of each 6-day irrigation cycle and the day following. Irrigation on the 6th day was applied at 0400 (EST) to permit changes in the response to the irrigation to be followed. Samples for water potential were taken

[illegible] sample was the entire terminal [illegible] from the topmost leaf to have just emerged from the meristem (7th leaf for the 1st cycle and the 10th leaf for the 2nd cycle). The second and third samples came from the base and tip of the next oldest leaf. These latter two samples consisted of 3 leaf discs obtained with a 6 mm diameter leaf punch

1983. To identify changes in leaf turgor and wall yield threshold at different leaf positions, turgor potentials were measured on leaves located at the 6th through 15th nodes of field-grown, droughted and well-watered soybeans. On 4 June, 1983, a 30 x 100 m plot was seeded in a manner similar to that described for the work conducted in 1982 (see Chapter II). All crop establishment and management practices were also the same with the exception that half the plot was well watered, being irrigated with 20 mm water during periods of rainfall insufficient to meet evapotranspiration and the other half subjected to natural droughts throughout the season. Throughout the growth of the crop, leaf samples were taken from both treatments for the determination of turgor potential at random times throughout the day. Effort was made to sample leaves having a variety of areas. From each plant, the terminal leaflet from the topmost leaf to have emerged from the meristem was sampled. The second and third samples were taken from the 2 leaves which subtended the first sample. Occasionally, fully expanded, mature leaves were sampled on plants used for the first three samples. Leaves which were larger than 25 cm^2 were sampled by means of a leaf punch as described earlier. Turgor potential measurements were performed as stated above, except

the psychrometers containing leaf tissue were permitted to equilibrate for 5 h before total water potentials were read.

1984. Leaf turgore and wall yield thresholds were measured every hour over 4 separate nights to assess more accurately these variables. Four adjacent 15 x 30 m plots were seeded on 2 April, 1984. Crop and field management was similar to that described for the 1982 experiment with the exception that Telone (1,3-dichloropropene) was incorporated into the field on 13 March to reduce nematode populations. All plots were irrigated by overhead sprinklers every second day with 20 mm of water unless otherwise indicated.

On 2 separate occasions in 2 separate plots, drought was imposed by withholding irrigations for 9 or 10 days. Total and osmotic leaf water potentials and areas of the leaves sampled were measured during each of the 4 experimental periods. Water potential measurements were made hourly and commenced at 1700 (EST) and were completed the next day at 1100 (EST). The first plot was droughted between the 3rd and the 13th of May. Water potentials and leaf areas were measured on 11 and 12 May. In the second plot, a well-watered treatment, leaf water potentials and leaf areas were measured on 21 and 22 May. The third plot was droughted between 24 May and 2 June. Leaf water potentials and leaf growth were measured in this plot on 31 May and on 1 June. The fourth plot was well-watered and was sampled for leaf water potential and leaf growth on 12 and 13 June. The droughted plots were irrigated as were the well-watered ones subsequent to the experimental period.

The procedure for measuring leaf water potential was identical to those used in 1982 with the exception that total leaf water potentials were recorded after allowing the leaf tissue to equilibrate for 5 hr. For the first three experimental cycles, three leaf sizes were sampled with three replications for a total of nine water potential samples each hour. Four replications of two leaf sizes were sampled during the fourth experimental cycle for a total of eight samples per hour. Care was taken to sample specific leaf sizes based upon the length of the terminal leaflet.

In a separate experiment, an unused portion of an adjacent plot was split into an irrigated and a droughted plot, each approximately 10 x 15 m. The irrigated plot received 20 mm water per application with an overhead irrigation sprinkler whenever rainfall did not meet evapotranspirational requirements. The droughted plot was subjected to natural rainfed conditions which was marked by short periods of drought lasting no more than 7 days. Three times between 1 and 12 August, 4 to 7 leaf water potential samples were gathered from each plot each day just prior to sunrise. Water potential samples were taken from the smallest, most recently emerged leaf from the meristem and also from the most recent fully expanded leaf. Procedures for the measurement of turgor potential were as stated above (1984 experiment).

Results

1984

Experiments conducted in 1984 were carried out to more carefully evaluate the water yield threshold of droughted and well-watered field-grown soybean leaves by increasing the number of measurement periods during the night and early morning when peak turgors are generally established in plants. This would insure that maximum turgors attained in well-watered, growing leaves would correspond to the yield threshold.

Leaf turgors from droughted plants (11-12 May) are plotted with respect to time of day in Fig. 4.1 for mature (4th leaf) and growing leaves (6th and 7th leaves). Mature leaf turgors were at their lowest values of approximately 0.20 MPa at 1800 (EST) and increased throughout the night to a maximum of 0.73 MPa by the following morning (Fig. 4.1a). Turgors in mature leaf tissue will never reach their yield threshold value since to do so would instigate growth. These turgors are consistent with values measured on mature, fully expanded Sillaxi soybean leaf tissue (Bennett, 1985 unpublished results). Turgors from the expanding 6th leaf, plotted in Fig. 4.1b, did not exhibit diurnal changes typically observed in mature leaves, instead they tended to remain steady at approximately 0.43 MPa between 1800 (EST) and 0500 the following day after an initially low turgor of 0.05 MPa at 1700 (EST) on the first day. The highest turgor, 0.5 MPa, was measured at approximately 0400. Turgors for the 7th leaf, the

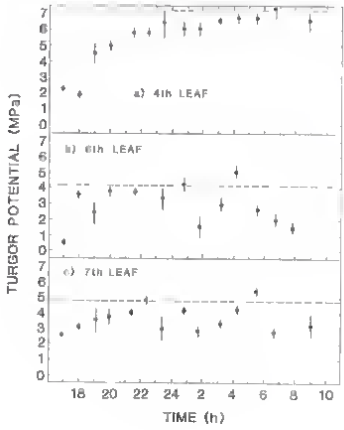

Figura 4.1 Diurnal turgor potentials of the 4th, 6th and 7th leaves from droughted plants, 11 - 12 May, 1964. Dashed horizontal line indicates the steady maximum turgor potential. Values are means ± S.E.

[illegible] associated with mature leaf tissue (Fig. 4.1c). Instead, a steady maximum leaf turgor of approximately 0.48 MPa was maintained throughout the night and early morning.

A second drought cycle (12-13 June) was used to obtain data to evaluate further leaf turgor of mature and expanding leaf tissue. Leaf turgors are plotted in Fig. 4.2 with respect to time of day for the mature leaf (12th), and the two youngest growing leaves (14th and 15th). At 1700 (EST), mature leaf turgors (Fig. 4.2a) were approximately 0.30 MPa and increased throughout the night to a maximum of 0.80 MPa prior to sunrise. The 14th leaf initially had a turgor similar to that measured in a mature leaf at 1700 (EST) (0.28 MPa) but during the night attained a maximum turgor of 0.57 MPa (Fig. 4.2b). Leaf turgor of the 15th leaf was approximately 0.16 MPa at 1700 (EST) and increased slightly throughout the night (Fig. 4.2c). Maximum leaf turgors were 0.40 MPa during the night, although these values were measured temporally adjacent to turgors averaging 0.18 MPa. Similar to the first drought cycle, growing leaves did not show as large a change in turgor during the transition from day to night as did the mature leaf.

Leaf turgors from leaves of well-watered plants were also evaluated on several occasions. Leaf turgors from 21-22 May, 1984 are plotted with respect to time of day in Fig. 4.3 for the 6th leaf (mature) and for several growing leaves (8th and 10th leaf). Mature leaf turgors at 1700 (EST) were 0.48 MPa, rose to a maximum of 0.90 MPa at 0200, and then dropped gradually to 0.30 MPa in the morning on

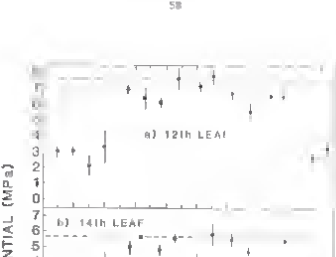

Figure 4.2 Diurnal turgor potentials of the 12th, 14th and 15th leaves from droughted plants, 12 - 13 June, 1984. Dashed horizontal line indicates the steady maximum turgor potential. Values are means ± S.E.

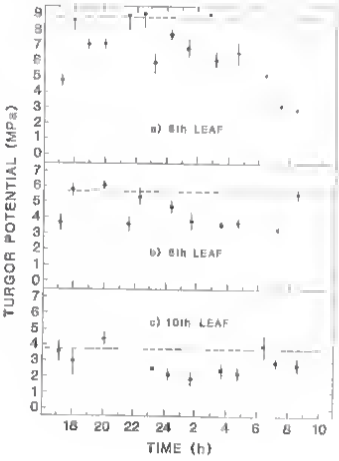

Figure 4.3 Diurnal turgor potentials of the 6th, 8th and 10th leaves from well-watered plants, 21 - 22 May, 1984. Dashed horizontal line indicates the steady maximum turgor potential. Values are means ± S.E.

[illegible] Turgors for the expanding 8th leaf did not exhibit any definite pattern throughout the course of the evening and morning but turgors generally centered around 0.41 MPa over the entire period (Fig. 4.3b). Likewise, the emerging 10th leaf did not display any large change in turgor during the transition from day to night and maintained an average turgor of approximately 0.26 MPa (Fig. 4.3c).

Leaf turgors from mature and expanding leaves (small) on well watered plants were again measured on 31 May and 1 June, 1984, and are plotted in Fig. 4.4. Only 2 classes of leaves were sampled to increase each sample size. Mature leaf turgors at 1700 (EST) were 0.34 MPa and increased throughout the night to a high of 0.55 MPa before returning to 0.30 MPa on the morning of 1 June (Fig. 4.4a). Turgors from small leaves did not exhibit any changes during the sample period and generally remained around 0.20 MPa with a high value of 0.25 MPa at 0200 (EST) (Fig. 4.4b).

A second experiment was conducted to obtain more data on the leaf wall yield threshold during a time when turgors should approach their maximum values just prior to sunrise (0500). At that time, the turgor potential of growing leaf tissue should exceed the leaf wall yield threshold. Under these circumstances and when measured in a vapor pressure psychrometer, the turgor potential should fall to the wall yield threshold. Leaf turgors (mean $\pm$ SE) for well-watered young and mature leaves were 0.30$\pm$0.06 and 0.85$\pm$0.11 MPa, respectively. Predawn turgors of the smallest droughted leaves were 0.43 $\pm$0.06 MPa and droughted mature leaves had turgors of 0.68$\pm$0.17 MPa. An increase

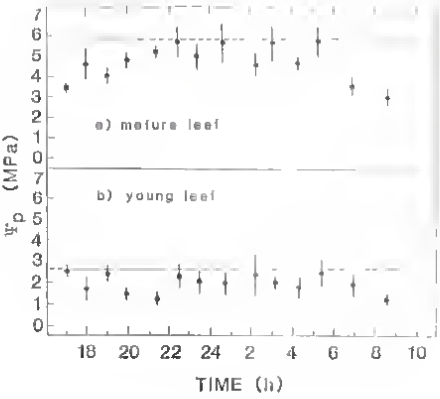

Figure 4.4 Diurnal turgor potentials of mature leaves and leaves recently emerged from the meristem from well watered plants, 31 May through 1 June, 1984. Dashed horizontal line indicates the steady maximum turgor potential. Values are means ± S.E.

turg[illegible] small leaves relative to those mature well-watered leaves indicates that these maximum turgors may be [illegible] wall yield thresholds. A comparison of the lower turgor in mature droughted leaves relative to well-watered ones puts into perspective the severity of drought

1983

Turgors of leaves gathered through the season and at various times of the day are plotted relative to the leaf area of the sampled leaf in Figs 4.5 for well-watered and droughted treatments. Drought reduced mature leaf turgors more than those from well-watered plants. The upper limit of turgor was approximately 0.3 MPa in small well-watered leaves (Fig. 4.5a) while it increased to approximately 0.5 MPa in small leaves from the droughted treatment (Fig. 4.5b). The higher turgors measured in small leaves of droughted plants were obtained during a night when turgor probably increased due to increased water uptake and a drop in transpiration. The higher turgors may have developed because cells were inhibited from expanding by a higher wall yield threshold. These results are consistent with the data given for the 1984 experiments in that the leaf wall yield threshold increased for the young, topmost leaves of droughted plants.

1982

Leaf turgors of irrigated (irrigated every 2 days) and droughted (irrigated every 8 days) plants are plotted against the time of day in Fig. 4.6 and Fig. 4.7 for the experimental period of 20-21 May, 1982.

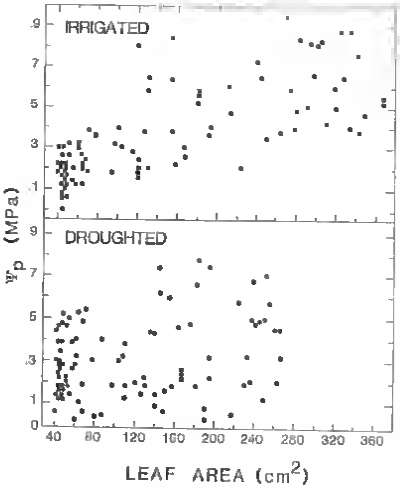

Figure 4.5 Leaf turgor potential from droughted and irrigated plants expressed as a function of leaf area, July and August, 1983.

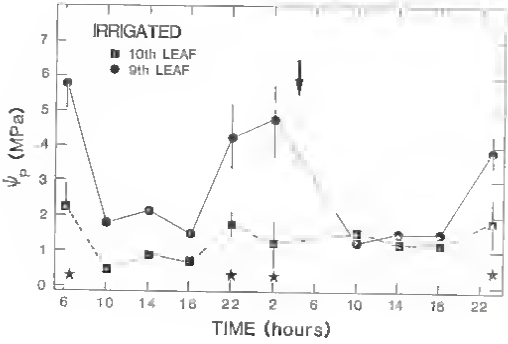

Figure 4.6 Diurnal turgor potentials from the 9th and 10th leaves from plants irrigated every 2 days, 20 - 21 May, 1982. Arrow indicates irrigation. Values are means ± S.E. A star (*) indicates significant differences at the (alpha=.05) level.

Figure 4 7 Diurnal turgor potentials from the 9th and 10th leaves from plants irrigated every 8 days, 20 21 May, 1982. Arrow indicates irrigation. Values are means ± S E. A star (*) indicates significant differences at the (alpha= 05) level

This period was just prior to and following [redacted] from drought for the plot irrigated every 8 days. The treatment irrigated every 4th day was not presented since these data were, for the greater part, quantitatively intermediate between the well-watered and droughted treatments and in general, not statistically different from the other two treatments. Data for the two locations on the 9th leaf (base and tip) were combined in all treatments since their sample averages were not significantly different.

Turgors of the irrigated 10th leaf were stable throughout the entire experimental period and averaged 0.13 MPa (Fig. 4.6). Turgors of the irrigated 9th leaf displayed diurnal changes probably associated with changes in temperature and solar radiation. Leaf turgors reached a high of approximately 0.45 MPa during the early morning and dropped to 0.13 MPa between 1000 and 1800 (EST).

In contrast to the behavior of the 9th and 10th leaves of the irrigated treatment (i.e. oscillatory versus stable turgor, respectively) the droughted 9th and 10th leaves were synchronized with respect to their turgors throughout the period of drought (Fig. 4.7). Prior to irrigation, turgors between 0600 (20 May) and 0200 (21 May), were similar for the 9th and 10th leaf. However, subsequent to irrigation (after 0400), the turgor of the 9th leaf rose to approximately 0.75 MPa while the turgor remained at approximately 0.23 MPa in the 10th leaf.

Discussion

Interpretation of Maximum Turgor in Growing Leaves

Wall yield thresholds may be distinguished from leaf turgor potential data with more precision at night. During the night, when plants generally begin to improve their water status, turgor rises in response to the increased availability of water. This ensures that, for at least part of the night the turgor potentials will exceed the minimum turgor required for growth to occur (wall yield threshold). If samples are removed from growing leaves at that time, the initial turgor will drop to the wall yield threshold. These values should then correspond to the highest turgors observed during the night. In droughted leaves, whose turgors may not be higher than the wall yield threshold, evaluation of the wall yield threshold then may not be possible. During the experiments of 1984, mature leaf turgor of droughted plants were only 0.1 to 0.2 MPa lower than turgor measured in mature leaves from well-watered plants. This would indicate that despite 8 days of drought, leaf turgor was able to recover to a large extent prior to sunrise. Accurate measurement of the wall yield threshold was possible at that time in those droughted plants.

Wall Yield Thresholds in Leaves of Well-Watered Plants

In every year of experimentation, turgor potentials of small leaves from well watered plants were stable throughout the night at values lower than those measured in mature, nongrowing leaves. For well-watered plants, the maximum leaf turgor (wall yield threshold) of

small l[illegible] ranged from 0.13 to 0.40 MPa while the maximum value for mature leaves ranged from 0.55 to 0.90 MPa (Table 4.2). This range of wall yield thresholds for growing leaves of well-watered plants includes the value calculated from the field data (0.24 MPa) of Bunce (1977).

Wall Yield Threshold in Response to Drought

A major goal was to observe the response of wall yield threshold to drought. It has been well recognized that in response to drought, turgor potential in mature, fully expanded soybean leaves declines. Lower turgor potentials were observed in droughted, mature leaves than in mature leaves from wall-watered plants during every experimental period. However, the turgors of small, growing leaves were higher in droughted than in well-watered treatments in both the 1983 experiment and during the nocturnal and early morning measurements during 1984. Maximum turgors of droughted small leaves ranged from 0.40 to 0.51 MPa while leaves from well-watered plants had maximum turgors in the range of 0.20 to 0.30 MPa (Table 4.2).

What appears as a discrepancy in the response of small leaves to drought, from a standard interpretation of turgor potential in mature leaves, is actually a key to understanding the response of the wall yield threshold to drought. That turgors for well watered small leaves equals wall yield thresholds was discussed above. In droughted, growing leaves which had turgors above the wall yield threshold for some portion of the night (as discussed earlier), the cell walls stiffened in response to drought, so that more turgor would

2 Maximum turgor and normalized leaf area of droughted and irrigated soybean leaves for experiments conducted between 1982 and 1984.

t	Leaflet position	Norm leaf area	Turgor, MPa
D", 1982	9	0.27	0.45
D 1982	10	0	0.51
D 1982, post irrig.	9	0.27	0.75
D 1982, post irrig	10	0	0.23
I, 1982	9	0.27	0.45
I, 1982	10	0	0.13
I 1982	9	0.27	0.39
I, 1982	10	0	0.19
D, 1983	small[y]	0	0.5
I, 1983	small	0	0.3
D, 1984	4	1.0	0.73
D, 1984	6	0.30	0.42
D, 1984	7	0	0.48
D, 1984	12	1.0	0.80
D, 1984	14	0.27	0.57
D 1984	15	0	0.40
I, 1984	6	1.0	0.90
I, 1984	8	0.30	0.41
I 1984	10	0	0.26
I, 1984[x]	small	0	0.25
I, 1984	mature	1.0	0.55
I, 1984[w]	small	0	0.30
I, 1984	mature	1.0	0.89
D, 1984	small	0	0.43
D, 1984	mature	1.0	0.88

[z] D: Droughted, I: Irrigated

[y] Leaves which recently emerged from the meristem, nodes 6 through 15.

[x] Night-time temperatures dropped to 9°C.

[w] 11 12 August.

[illegible]rred [illegible]r. Stated another way, the wall yield threshold increased in droughted, growing leaves. Maize and Jing (1984) who utilized the Shardakov technique for measuring water potentials, also observed an increase of approximately 0.2 MPa in the wall yield threshold in droughted maize leaves (Zea mays). In droughted pea stems, Cosgrove (1986) reported no change in wall yield threshold, as measured by a stress relaxation and pressure probe technique. However, in his experiment, drought was imposed over a short time compared to the length of drought experienced in the field experiments (up to 8 days). Several days of drought might be required to appreciably increase the wall yield threshold. Furthermore, since wall yield threshold appears to be sensitive to the growth environment (Bunce, 1977), Cosgrove's results are not necessarily contradictory to the results in the present work.

An opportunity to observe the response of wall yield threshold after release from drought was available during the experiment of 20 - 21 May, 1982. After irrigation to a droughted plot by 0400 on 21 May, turgor of a small, growing leaf changed abruptly from diurnal oscillations, having a maximum of 0.51 MPa, to a steady value of approximately 0.25 MPa. The latter value of turgor was similar to that of small, growing leaves from well watered plants. Once irrigated, formerly droughted plants should have been able to rehydrate sufficiently for turgor to exceed the wall yield threshold. Evidence that this was the case lies in the fact that leaf turgor exceeded 0.7 MPa in the 9th leaf by 1000. Therefore, leaf turgor was higher than the wall yield threshold in the small, formerly droughted

[illegible] equilibration. Therefore, the value recorded after equilibration equaled the wall yield threshold. Whatever process caused wall stiffening in response to drought, reversed rapidly upon rewatering.

Wall Yield Threshold and Leaf Area

Maximum turgors and area of leaves (expressed on a normalized basis) sampled for turgor potential are presented for each of the experiments conducted between 1982 and 1984 (Table 4.2). Linear regression analyses were performed to correlate maximum turgors (wall yield thresholds, WYT) with normalized leaf area (NLA), for both well-watered and droughted leaves. This resulted in equations 4.4 and 4.5 for well watered and droughted leaves, respectively.

$$WYT = 0.57*NLA + 0.30 \quad (r^2 = 0.94) \qquad (4.4)$$

$$WYT = 0.24*NLA + 0.46 \quad (r^2 = 0.86) \qquad (4.5)$$

These equations support the concept that wall yield threshold increases with increasing leaf area. The relationship between wall yield threshold and leaf area supports an important relationship established from an examination of the wall relaxation equation: wall yield thresholds should never be exceeded by turgor in mature leaves, for to do so would require that they grow. This was also confirmed by Van Volkenburgh and Cleland (1986), who could detect no wall relaxation in mature bean leaf tissue. These equations emphasize that, at maturity, leaves seldom experience turgor greater than 0.89 or 0.70 MPa in well watered and droughted leaves, respectively.

Conclusion

Wall yield threshold was effectively measured with vapor pressure psychrometers at night and before sunrise in well-watered plants and in droughted plants which were able recover from drought during the night. Young leaves from well-watered plants had wall yield thresholds of approximately 0.3 MPa while similarly sized leaves from plants droughted for 8 days rose to 0.5 MPa. Equations were developed which related the wall yield thresholds to normalized leaf area for well-watered and droughted leaves

CHAPTER V
EVALUATION OF THE RELATIVE LEAF GROWTH RATE AND WALL EXTENSIBILITY IN FIELD-GROWN SOYBEAN LEAVES

The wall relaxation equation (eqn. 5.1), was proposed in Chapter

$$(1/V)dV/dt = \phi(P-Y) \qquad (5.1)$$

IV to be sufficient to describe leaf expansion. Turgor potential, P, and wall yield threshold, Y, were evaluated in field-grown, droughted and irrigated soybeans in Chapter IV. To evaluate all of the variables of the wall relaxation equation in leaves of field grown soybeans, the leaf relative growth rate, $(1/A)dA/dt$ (where the leaf area, A, is set equal to the volume, V, multiplied by a constant, from eqn. 5.1), and the wall extensibility, ϕ, need to be measured or estimated. Therefore, a modified form (eqn. 5.2) of the wall relaxation equation was employed to study leaf growth:

$$(1/A)dA/dt = \phi(P - Y) \qquad (5.2)$$

A goal was to examine the influence of drought on the leaf relative growth rate and the wall extensibility. Leaf relative growth rates were calculated from changes in the leaf area over various time intervals.

Despite general acceptance of the concepts inherent in the cell wall relaxation equation over the last decade, there have been very few studies which attempted to measure wall extensibility. To date, there have been no field studies of this parameter despite the fact that it could, in theory, control the growth of plant organs

Although Va[illegible] and Cleland and [illegible] did not evaluate wall extensibility, they concluded that since the effective turgor, (P - Y), was not related to the growth rate, the wall extensibility must control expansion of growth chamber grown bean (Phaseolus vulgaris L.) leaves. However, calculation of wall extensibility from their data indicate that wall extensibilities would need to be several orders of magnitude higher than values obtained by other, more direct techniques. Another estimate of wall extensibility (Boyer et al., 1985) may be plagued by inadequate equilibration times in the measurement of wall yield threshold, so these values were also five-times higher than values reported by Cosgrove (1985). Boyer et al. (1985) made measurements of the wall yield threshold in less than 30 minutes after removing the material from its source of water. Cosgrove (1985) has shown that the decline in the turgor potential to the wall yield threshold requires at least several hours. Cosgrove (1985), who employed a micro pressure probe, developed a dynamic measure of wall extensibility in dark-grown pea (Pisum sativum) stem cells and found wall extensibility values essentially indistinguishable from values measured with a steady-state approach. Since so few experiments have been carried out on this parameter and because cell wall properties are greatly influenced by growth environment, there is a need to evaluate the wall extensibility under field conditions.

To assess the relative growth rate and wall extensibility of soybean leaves, field experiments were conducted over several years on droughted and irrigated, soybean plants. Leaf relative growth rates

[illegible] leaf area (expressed on a normalized basis) for [illegible] having areas greater than 20% of their final value. Estimated leaf wall extensibility was found to vary between 0.08 and 0.40 MPa^{-1} h $^{-1}$. It was not possible to analyze effectively the data to determine the influence of drought on the leaf wall extensibility.

Materials and Methods

Leaf growth data for this analysis were obtained from experiments performed in 1982 and 1984. The cultural conditions for the plants grown in these experiments were presented in Chapters II and III.

Leaf Relative Growth Rate

In 1982, the length and breadth of terminal leaflets were measured with a millimeter scale and converted to leaf area by means of equation (2.3). Similarly, in 1984, only leaflet lengths were measured and converted to leaf area with equation (3.1). Leaflet dimensions were measured periodically in the morning on the topmost 5 terminal leaflets of 10 plants in plots irrigated every 2nd, 4th and 8th day (1982). The time interval between measurements ranged from 16 to 49 hours. From the changes in leaf area and the appropriate time interval, the leaf relative growth rate was calculated. At the end of the experimental period, leaves which had been used for area determinations were allowed to complete their growth under well-watered conditions. Upon full expansion, they were remeasured and these final areas were used to express previous leaf areas on a normalized basis.

Relative growth rate was measured again in May and June, 1984, twice in an irrigated plot and twice in one which had not been irrigated for 8 days. The time interval between measurements was reduced to 16 hours. The average value of final leaf area at a particular node in a plot was used to calculate normalized leaf area.

Leaf relative growth rate was again measured in July 1984 on a second irrigated plot at approximately every 6 h over a 4 day period. Plot averages of final leaf area were again used to calculate the normalized leaf area.

Leaf Wall Extensibilities

The wall relaxation equation for leaves (eqn. 5.2), can be rewritten to solve explicitly for wall extensibility.

$$\phi = (1/A)dA/dt \;/\; (P - Y) \qquad (5.3)$$

Extensibility is then a function of the leaf relative growth rate, the leaf turgor and the leaf wall yield threshold. Since each term on the right side of eqn. 5.3 was measured, it was possible to then estimate the leaf wall extensibility.

Results and Discussion

Leaf Relative Growth Rate

Leaf relative growth rate data collected in 1984 from irrigated and 8-day droughted treatments are plotted in Fig. 5.1 and 5.2, respectively. Since field plot averages of fully expanded leaf areas

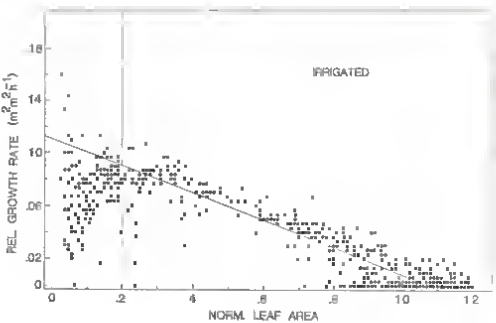

Figure 5.1 Leaf relative growth rates as a function of their normalized areas for irrigated plants, 1984

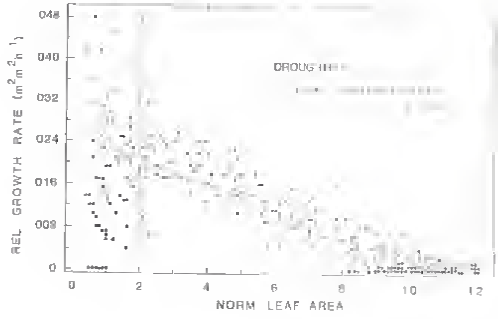

Figure 5-2 Leaf relative growth rates as a function of their normalized areas for droughted plants, 1984

were used in calculation of normalized leaf areas. Normalized leaf areas deviated from unity. As discussed in Chapter III, two groups of data may be delineated; small leaves to the left of the perpendicular line at 0.2 units of normalized leaf area and more mature leaves to the right. The latter group of data extends from the highest leaf relative growth rate to the lowest along a line whose slope decreases as it approaches unity on the abscissa. From Chapter III and references therein, it was shown that a significant number of cells are undergoing division in leaves which are still in the exponential phase of growth. Significant deviation from the expected growth rate should result for leaves with a high percentage of dividing cells, since cells, while they are dividing do not expand (Freisner, 1927).

Data collected in 1982 and 1984 were similarly analyzed by linear regression using only data from leaves having normalized areas greater than 0.2. The slopes of the unfolded leaf relative growth rate for plants irrigated every 2, 4 or 8 days in 1982 are presented in Table 5.1. There is a general lack of statistically significant difference between the treatments irrigated every 4th or 8th day when the relative growth rates are compared on any given date. As the drought progressed in severity from the first to the eighth day, the slopes did not change in any regular fashion. However, when the slopes were averaged over all measurement periods, they significantly increased with an increase in the amount of irrigation.

Table 5.1 Slopes of regression of leaf relative [illegible] rates vs normalized leaf areas for pots irrigated every 2, 4, or 8 days, 1982.

Day of year	Irrigation interval (days) 2	4	8	Time interval (h)
		slopes		
127 128	-0.041a	-0.045a	-0.035a	49
129 130	-0.075a	-0.040b	-0.025c	17
130 131	-0.021a	-0.017b	-0.014b	24
131 132	-0.035a	-0.028b	-0.025b	26
132 133	-0.030a	-0.025a	-0.027a	27
133 134	-0.023a	0.024a	0.028a	21
134 135	0.045a	0.032b	-0.031b	30
135 136	-0.028a	0.030a	-0.036b	18
136 137	0.024a	0.025a	0.020a	32
137 138	-0.052a	0.034a	-	17
138 139	-0.032a	0.035a	-	29
139 140	-0.050a	0.026a		16
140 141	-0.035a	-0.039a	-0.021b	24
141 142	0.030a	-0.040b	-0.035c	37
Total[a] means ± S.E.	0.037±0.004a	0.031±0.008b	0.027±0.007c	

[a]Row values followed by the same letter are not significantly different at the (alpha=0.05) level according to an analysis of covariance

There are several possible explanations of the poor coupling between these slopes and the effect of drought on relative leaf growth rate on a daily basis. First is the manner in which data were collected and subsequently presented. All leaf relative growth data in this chapter are termed cross sectional data (Kaufmann, 1981), which means that growth data are recorded from individual leaves of different sizes at one time. It is equivalent to recording the growth of individuals at different times along their growth trajectory. The assumption implicit in utilizing cross-sectional data, as is done in this chapter, is that all individuals follow the same growth trajectory. Data presented in Chapter III indicate that small leaves respond differently than large leaves to drought. If so, then the leaf growth data must be viewed within the limitations inherent to cross-sectional data.

A second difficulty with the interpretation of leaf relative growth data stems from the rapid changes that occur in both the environment and in the leaf itself during the time interval over which growth measurements are made. This may have led to faulty estimations of the wall extensibility by Van Volkenburgh and Cleland (1986), who measured effective turgor and relative growth rate but once over a 12 h period. These variables may change even in a constant environment due to cycling of stomata or control exerted by internal clocks.

In 1982, leaf growth measurements were done over time intervals ranging from 16 to 40 hours. If leaf growth is dependent upon the water relations parameters described above, then it is obvious that growth measured over even a 16-hour period (May and Juma, 1984) will

provide numerous opportunities for leaf turgor to show wide fluctuations. To reduce the influence of this problem, an attempt was made to reduce the time interval between growth measurements. Leaf relative growth rates were calculated based upon a 6 h time interval in July 1984 (Table 5.3). The coefficient of determination from linear regressions of relative growth rate versus normalized leaf area, did not improve considerably with a decrease in the time interval between measurements from 16 h (May and June, 1984; Table 5.2) to 6 h (July, 1984; Table 5.3).

The failure to reduce relative growth rate variability may have been due to changes in leaf turgor over time periods as short as seconds. There is ample evidence that several minutes are required before large changes are evident (as when a cloud passes and casts a shadow over a crop). Temperature may also influence the relative growth rate of soybean leaves. Soybean leaf growth has been shown to have a base temperature of 9 - 10°C in several studies (Heakath et al., 1973; Sinclair, 1984). During the night of 31 May and the morning of 1 June, air temperature dropped to 9°C and probably resulted in the low growth rate of the larger leaves observed during that experimental period (Table 5.2).

In each treatment, leaf relative growth rate data were averaged over 14 measurement periods in 1982 (Table 5.1). These mean values were used to develop equations which relate leaf relative growth rate of leaves having normalized areas greater than 0.2 to the normalized

Table 5.2 Slope [illegible] the leaf [illegible] growth [illegible] vs [illegible] leaf [illegible] greater than 0.7 (1984).

Treatment	Day of [illegible]		slope	intercept	N	r^2
Droughted	132	137	0.026	0.026	654	65[z]
Well watered	142	143	0.018	0.024	800	73
Well watered	152 -	153	-0.016	0.011	287	20
Droughted	158	159	-0.019	0.019	108	60

[z] All coefficients of determination were significant at the (alpha=.05) level.

[illegible] and [illegible] the leaf relative growth r[illegible] normal[illegible] leaf areas greater than 0 [illegible] 1984)

Day of year	Hour interval	slope	intercept	r
198	0000 - 0600	-0.046	0.044	.61[z]
198	0600 - 1200	-0.033	0.029	.64
198	1200 - 1800	0.018	0.017	.26
198	1800 - 2400	0.055	0.054	.71
199	0000 - 0900	0.043	0.043	.58
199	0900 - 1200	-0.031	0.025	.20
199	1200 - 1800	-0.020	0.021	.53
199	1800 - 2400	-0.035	0.040	.63
200	0000 - 0600	0.034	0.042	.53
200	0600 - 1200	0.022	0.024	.65
200	1200 - 1800	0.026	0.030	.73
200	1800 - 2400	-0.014	0.020	.51
201	0000 - 0600	0.030	0.035	.68
201	0600 - 1800	-0.021	0.022	.77
201	1800 - 2400	0.006	0.011	.30
202	0000 - 0600	-0.025	0.028	.81
Totals mean ± S E		.029±0.013	0.030±0.012	

[z] All coefficients of determination were significant at the (alpha=.001) level.

)ef area (NLA). This resulted in the following equations for well watered (equ. 5.4) and droughted (equ. 5.5) plants, respectively.

$$(1/A)dA/dt = -0.037*NLA + 0.037 \quad (5.4)$$

$$(1/A)dA/dt = -0.027*NLA + 0.027 \quad (5.5)$$

Evaluation of Leaf Wall Extensibility

Equation 5.3 was used to determine the leaf wall extensibility for a range of mature leaf turgor potentials by substituting equations derived for the leaf relative growth rate ((1/A)dA/dt) and the leaf wall yield threshold (Y). Equation 5.3 was solved separately for well-watered and droughted leaves. Expressions derived for the leaf relative growth rate of well watered (equ. 5.4) and droughted (equ. 5.5) leaves were used in equation 5.3. The following wall yield threshold equations for well watered and droughted leaves (eqns. 4.4 and 4.5, respectively) were also used in equation 5.3 to estimate wall extensibility.

$$WYT = 0.57*NLA + 0.30 \quad (5.6)$$

$$WYT = 0.24*NLA + 0.46 \quad (5.7)$$

Due to the dependence of leaf relative growth rate and leaf wall yield threshold on normalized leaf area, leaf wall extensibilities could only be solved with respect to a range of normalized leaf area.

Plots of the estimated leaf wall extensibility as a function of normalized leaf areas for a range of leaf turgor potentials are presented in Fig. 5.3 for the well-watered case. An essentially identical plot was obtained using equations developed from droughted leaves so it is not presented.

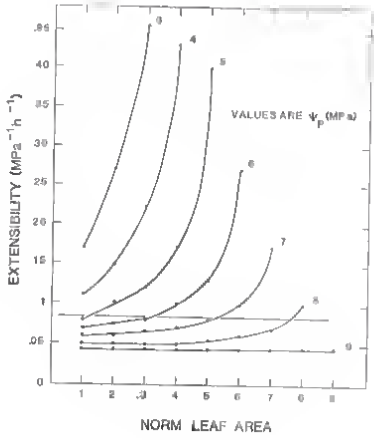

Figure 5-5 Estimated leaf wall extensibility as a function of the normalized leaf area and for various leaf turgor potentials. Horizontal line corresponds to a wall extensibility of 0.08 MPa^{-1} h^{-1}

To identify a particular leaf wall extensibility from Fig. 5.3, the leaf turgor must be known. The initial turgor potentials (pressure relaxed) were estimated by assuming that the total and osmotic water potential gradients were minimal between the topmost growing leaves and the next fully expanded leaf. Turgor potentials in all growing leaves, then, should equal those measured in fully expanded leaves. Turgor potentials of fully expanded mature leaves were measured during May and June, 1984 in both well watered and droughted plants (Figs. 4.1 - 4.4). Average mature leaf turgor potentials during the 16 h measurement period were 0.70 and 0.55 MPa for the well-watered treatment (21 - 22 May) and the two droughted treatments (11 - 12 May and 12 - 13 June), respectively. From Fig. 5.3, it can be seen that at leaf turgor equal to 0.70 MPa (well watered case), leaf wall extensibilities ranged from 0.06 to 0.15 $MPa^{-1}\ h^{-1}$ for normalized leaf areas which ranged from 0.1 to 0.7. Leaf wall extensibilities from droughted mature leaves having an average turgor of 0.55 MPa, ranged from approximately 0.08 to 0.40 $MPa^{-1}\ h^{-1}$ for normalized leaf areas between 0.1 and 0.5. These lower values compare favorably with wall extensibilities of 0.08 $MPa^{-1}\ h^{-1}$ measured via dynamic relaxation and steady-state techniques (Cosgrove, 1985) on pea stems and other theoretical estimates (Cosgrove, 1981).

Conclusion

The wall relaxation equation (eqn. 5.2) was proposed to serve as a model of cell growth in leaves of droughted and irrigated soybean. Leaf relative growth rate was measured in well-watered and droughted

[illegible] leaf [illegible] with [illegible] including leaves of normalized areas less than 0.2, the slope of relative growth rate versus normalized leaf area was used in the cell wall relaxation equation to evaluate wall extensibility. Estimated wall extensibilities from field experiments (0.06 to 0.40 MPa^{-1} h^{-1}) were in reasonable agreement with a value of 0.08 MPa^{-1} h^{-1} reported by Cosgrove (1985). This strongly supports the ability of the cell wall relaxation equation to model changes in bulk populations of cells as exist in leaves.

CHAPTER VI
INDIVIDUAL SOYBEAN LEAF GROWTH MODEL

From a mechanistic viewpoint, growth and development of leaves under optimal or stressed conditions have not been understood, nor has there been much success in predicting these basic plant processes. Jones and Hesketh (1980) concluded, after developing a leaf growth model that more research was needed which would be "based on a morphogenic approach . and which would realistically synthesize the effects of stresses on the rate of initiation, growth and senescence of individual organs of a plant". Understanding growth and development of leaves is crucial to any effort to improve the yield potential of all crops, since it is the leaf, as the primary photosynthetic organ of plants, that defines the upper limit to yield formation. Therefore, a mechanistic model of leaf growth based upon the water relations and population dynamics of leaf cells was developed. The primary goal of this model was to assess the importance of leaf turgor, wall yield threshold, wall extensibility and other parameters thought to play a role in cell division on leaf growth. A second major objective was to determine the impact of drought through these factors on leaf growth.

There are few models in the literature which attempt to relate the effects of drought to the growth and development of individual leaves. Available models describing growth and development of individual leaves are computationally bulky, are based upon

numerous empirical coefficients, and employ [illegible] as the basis within the leaf as the basis for controlling growth (Charles-Edwards, 1979; Thornley et al., 1981). Water relations variables are generally ignored and morphological characteristics are not utilized as a basis for development, so the ability of these models to predict leaf growth during periods of stress is poor or non-existant.

A unique feature of the model presented in this chapter is the portrayal of leaf growth as a combination of cell production and their subsequent expansion. A central concept of this model is that, in the absence of other limiting factors to growth, such as low temperatures or poor nutrition, the division of cells is limited by the ability of each cell to expand to a minimum volume needed for cell division to occur. This requirement is based on the simple observation that cells do not continue to divide without subsequent increase in volume, for to do so, cells would quickly divide themselves into units having volumes smaller than most cell organelles. The treatment of leaf cell expansion is also unique in that the cell wall relaxation equation (eqn. 5.1) serves to set the rate of cell expansion as well as acting as a governor limiting the division of cells.

Model Development

Outline of Dicot Leaf Growth

Cell division in a dicot leaf originates from meristematic zones within a young leaf structure. Cells produced in the meristems increase in volume but at a rate much slower than expansion which occurs after meristematic activity has ceased (Sunderland and Brown,

1956). Once the leaf emerges from the meristem, differentiation of the epidermis, vascular, palisade and mesophyl cells has been completed. At this time, cell division no longer occurs within well defined meristematic zones but occurs throughout each cell layer. The soybean leaf growth model only considers cell expansion and division at that juncture and until leaf growth has stopped. Cell division proceeds at an exponential rate until the leaf blade has unfolded or, in some cases, several days after unfolding (Dale, 1976; see Chapter III). Cell division ceases completely when the leaf blade has attained approximately 75% of its mature area although many species are capable of continued cell division until full leaf blade expansion. Cell division of the palisade layer in soybean leaves may continue into the last quarter of leaf blade expansion (Lugg and Sinclair, 1981). Dale (1970) has predicted, based on cell division models, that leaves have the capacity for cell division beyond the time when division normally ceases. Unfortunately, no progress has been made in determining the reason(s) for when cells cease to divide nor what factors control final leaf size (Charles Edwards, 1979).

The factors controlling cell expansion have been proposed (Lockhart, 1965; Green et al., 1971; Cosgrove, 1981) and now serve as a basis for considerable research effort (Boyer et al., 1985; Cosgrove, 1986). The factors controlling cell expansion were discussed in detail in Chapter IV, however, little information exists as to the relationship between individual cell expansion and whole leaf growth. Dale (1964) has shown that the average cell volume in bean leaves increases with time and leaf size. The increase in cell

volume as the leaf grows serves as a central concept in the soybean leaf growth model. It links the two basic processes occurring in the leaf: cell division and cell expansion.

The structure of the soybean leaf growth model may be divided conveniently into cell expansion and cell division submodels. Each submodel will be described from a mathematical standpoint as well as a description of how each was implemented.

Mathematics of the Model

Cell expansion. A modified cell wall relaxation equation (eqn. 6.1) was employed to determine the rate of cell expansion:

$$V' = \phi [P(t) - Y(A)] \qquad (6.1)$$

where the relative expansion rate of a cell is V' ($um^3\ um^{-3}\ h^{-1}$), the cell extensibility is ϕ ($MPa^{-1}\ h^{-1}$), the time dependent cell turgor potential is P(t) (MPa) and the leaf area dependent cell wall yield threshold is Y(A) (MPa). Each cell in a leaf is presumed to expand at a rate determined by eqn. (6.1) regardless of its initial volume. Cell expansion requires that the turgor potential exceed the wall yield threshold, whereas the wall extensibility relates the relative rate of expansion to the effective turgor (P - Y) and plays no role in determining if expansion occurs.

A constant wall extensibility, $0.08\ MPa^{-1}\ h^{-1}$, is used throughout leaf growth since it was the value determined by Cosgrove (1985) on pea (*Pisum sativum*) stem cells and represents the lower range of estimated values for both droughted and well-watered soybean leaves (see Chapter V). Simulations will show that a constant wall

extensibility [illegible] to [illegible]
realistic leaf [illegible] with time

The wall yield threshold, [illegible], as a function of leaf area for well watered (eqn 6.2) and drought (eqn 6.3) leaves

$$Y(A) = 0.0021*A + 0.$$ [illegible]

$$Y(A) = 0.0009*A + 0.50 \qquad (6.$$

Equations (6.2) and (6.3) were obtained by dividing the normalized areas in eqns (4.4) and (4.5) by the final area of the 6th leaf (260 cm^2) from a well-watered plant. The intercepts of eqns (6.2) and (6.3) are termed the initial wall yield threshold since this corresponds to the wall yield threshold of leaves as they emerge from the meristem. These equations reflect three important relationships between the wall yield threshold and leaf growth. 1) the wall yield threshold increases with increasing leaf size, 2) drought increases the wall yield threshold above that for well-watered leaves and 3) the wall yield threshold serves to set the maximum leaf area attainable. When the wall yield threshold reaches a value corresponding to the highest leaf turgor, cell expansion ceases and the maximum leaf area is attained

Leaf turgor potential, P(t), is a function of the time of day (t, hour of day) and is set equal to equations (6.4) and (6.5) for well watered and droughted leaves, respectively.

$$P(t) = 0.3[1.9 + \sin(t+4)/12\ ] \qquad (6.4)$$

$$P(t) = 0.3[1.9 + \sin(t+4)/12\ ] - 0.25 \qquad (6.5)$$

The turgor potential reaches a maximum at 0200 and a minimum at 1400 for both equations. The maxima and minima of turgor potential are

0 87 and 0.27 MPa for well-watered leaves and 0.62 and 0.02 MPa for droughted leaves. The times of maxima and minima are not relevant to the success of the model in predicting final leaf area since it is only the daily integrated turgor potential which dictates final leaf area. However, to properly interpret simulations of diurnal fluctuations in growth rates, turgor potentials were simulated to reflect those measured under field conditions. The amplitude of the turgor potential (0.6 MPa) is the same for well-watered and droughted leaves. This was measured under field conditions and may reflect the ability of the entire plant to regulate water loss to certain physiologically imposed limits.

Cell division. An equation termed the minimum-volume division function (eqn. 6.6) was developed to link the process of cell division with cell expansion

$$S(t) = 12*t + 800 \qquad (6.6)$$

S(t) is a time dependent cell volume (um^3), where t is cumulative hours from the moment the leaf emerges from the meristem. At that time the leaf is presumed to have ceased well-defined meristematic activity. S(t) is more precisely defined as the cell volume which must be reached before the cell may divide. From eqn. (6.6) it may be seen that when a leaf emerges from the meristem (t=0), cells are required to have a volume of 800 um^3 to divide. Based on data presented in Fig. 3.7, the average cell volume at the time the leaf emerged from the meristem was 700 um^3. This average cell volume would then be insufficient for cell division to occur based on eqn. (6.6). The slope of eqn. (6.6), 12 $um^3\ h^{-1}$, establishes the rate of increase

[illegible] slope for the increase in the minimum volume required for division was chosen based on the final number of cells found experimentally for the 6th leaf (Fig. 3.7).

The minimum volume division function provides a means for terminating cell division in a realistic manner and thus limits final organ size inasmuch as cell number determines this latter value. As leaf growth progresses, the ability of the cells to expand decreases due to reduced effective turgor caused by an increase in the wall yield threshold. Cell division is terminated because the rate of cell expansion cannot keep pace with the minimum cell volume required for division established in eqn. (6.6). Equation (6.6) was used successfully in simulations of well-watered and droughted leaf growth. This suggests that drought may act to reduce cell division directly through an effect on the potential expansion rate of cells and not through an effect on cell metabolism directly linked to the division of cells.

Development of the minimum-volume division function. Before presenting the final soybean leaf growth model, a discussion of a preliminary model used to assist in developing the minimum-volume division function (eqn. 6.6) is presented. The purpose of the preliminary model was to determine the effect of an imposed exponential rate of cell division during early growth on average cell volume. The change in average cell volume with time obtained from simulations of leaf growth with the preliminary model became the basis for evaluating the coefficients of eqn. (6.6). Exponential cell

division rate was imposed in the model since most studies [illegible] earlier, found an exponential increase in the number of cells with time during early stages of growth.

The preliminary model uses a discrete boxcar approach to move cells towards a state of maturity, whereupon they cease to divide (Penning de Vries and van Laar, 1982). A FORTRAN listing of the code is presented in Appendix B. Each box, termed a volume average class size or 'VACS', contains a specific number of cells at any given time with an associated cell volume expressed in cubic microns. Based on data presented in Chapter III, a leaf emerges from the meristem with approximately 15 million cells, and the average cell volume is 700 um^3. Therefore, at the initiation of leaf growth, which for present purposes corresponds to leaf emergence from the meristem, each of the lowest 15 VACS's are filled with one million cells. The smallest VACS is 665 cubic microns and successive VACS's are incremented by 5 cubic microns. This resulted in an average initial volume of 695 um^3 $cell^{-1}$.

Cell division is simulated by taking a certain percentage of the total number of cells and doubling them. This percentage is established by an exponential coefficient equal to 0.004 and an initial cell population equal to 15 million, both values approximated from data presented in Fig. 3.7 for a well watered leaf. Once a cell has divided, it is reallocated into a new VACS based on its new halved volume.

After cells divide they are then expanded according to the modified wall relaxation equation (eqn. 6.1). Equation (6.2) is used to describe wall yield threshold and wall extensibility is set to a

[illegible] Equation [illegible] is used to define leaf turgor potential. As each group of cells is expanded they are shifted to a new VACS.

At each interval of time, the total leaf volume is calculated by summing the products of the number of cells in each VACS by that VACS's volume. Leaf volume was increased by 20% to account for air spaces. Leaf area was then calculated by dividing the leaf volume by an average leaf thickness of 150 um (Lugg and Sinclair, 1980).

The effect of exponential cell division on average cell volume during leaf growth is illustrated by the average cell volumes plotted against time (Fig. 6.1). Average cell volume increased linearly with time for approximately 94 h, then it declined to increasingly smaller volumes. The decline in average cell volume was due to the excessively high rate of cell division caused by exponential increase in the number of cells. Too many cells result in too small a volume for each cell. This supports observations first discussed in Chapter III, that the exponential increase in the number of cells is limited to 4 or 5 days after leaf emergence in many cases of leaf growth.

A linear regression analysis of the relationship between average cell volume and time (h) up to day 5 resulted in eqn. (6.8). This equation is termed the minimum volume-division function where V, is the leaf cell volume (um^3) and t is cumulative time (h).

$$V = 34*t + 800 \qquad (6.8)$$

This equation simply states that a cell must attain a minimum, time-dependent volume before it may divide. Initially (when the leaf has emerged from the meristem), cells must have a volume of 800 um^3 in

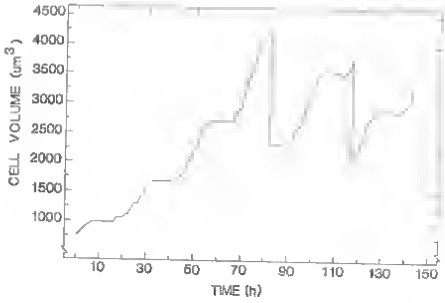

Figure 6 1 The change in the cell volume with time, for the 6th leaf simulated with a preliminary growth model.

[illegible] subsequent size [illegible] and the minimum volume [illegible] eqn. 6.6) to regulate cell division instead of the [illegible]ent exponential function

Informational diagram of the soybean leaf growth model. Computer implementation of the soybean leaf growth model is presented here to facilitate interpretation of the FORTRAN code presented in Appendix C A flow chart of the soybean leaf growth model is presented in Fig. 6.2 At each time interval, cells are expanded according to eqn. (6.1) using the appropriate values of turgor potential, wall yield threshold and wall extensibility. If cell volume meets the minimum volume established by eqn. (6.6) then a cell divides resulting in 2 daughter cells each of half the original cell volume At this point, the average cell volume is calculated and if it is 5000 um^3 or greater then leaf growth stops, otherwise leaf cells continue to expand and divide in the next time increment according to the controlling equations. Average cell volumes were approximately 5000 um^3 in the mature 6th leaf based on data from Fig 3.7. Leaf volume is calculated by summing the product of each cell class by its respective volume and multiplying by the appropriate constants to convert volume to leaf area

To achieve regular and smooth increases in the number of cells and leaf area, 25 cell classes are used. At the start of a simulation, each class is filled with 600,000 cells (i.e., an initial total of 15 million cells for the leaf). The first class of

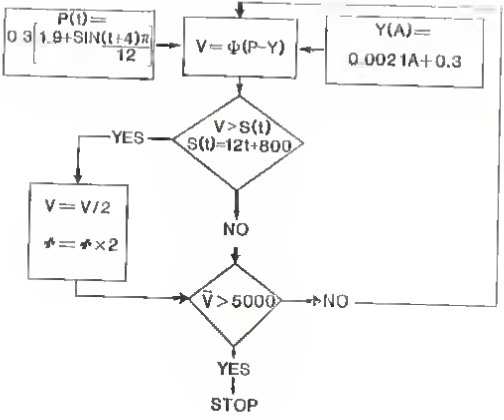

Figure 6.2 Flow chart of the soybean leaf growth model.

[illegible] was assigned an average volume of 400 um^3 and [illegible] cleese was 25 um^3 larger than the one which preceded [illegible] in an average cell volume of 700 um^3 per cell at th[illegible] growth

Simulation of Leaf Growth - Nominal Case

To facilitate interpretation of the model and o subsequent parameter analyses, the nominal values of the parameters and equation used to simulate growth of the 6th leaf of well-watered and droughted soybean plants are listed in Table 6.1 Simulations of growth of a well-watered leaf were performed as described earlier. Leaf area and cell number as a function of time were outputs of simulations using the parameters listed in Table 6.1

Simulated number of cells for a well-watered leaf are plotted in Fig. 6.3. The early increase in cell number was exponential-like, and the overall increase in cell number over time was sigmoidal. Cell division ceased at 360 h. Simulated cell number was essentially identical (r^2=0.981 to those measured in well-watered leaves (1983 data, Fig. 3.7).

Simulated change in area of the 6th leaf from a well-watered plant also is plotted in Fig. 6.3. Leaf area increased sigmoidally with time and terminated growth at 528 h. The simulated final leaf area was within 4% of the measured leaf areas (1983 data, Fig. 3.7).

Table 6.1 Nominal values and equations used in simulations of soybean leaf growth during well-watered and droughted conditions

Variable	Units	Well watered	Droughted
ϕ	$MPa^{-1}\ h^{-1}$	0.08	0.08
P	MPa	0.3(1.9 + sin(t+4) /12]	0.3(1.9 + sin(t+4) /12] .25
Y	MPa	0.0021*A + 0.3	0.00009*A + 0.5
S(t)	$um^3\ h^{-1}$	12*t + 800	12*t + 800

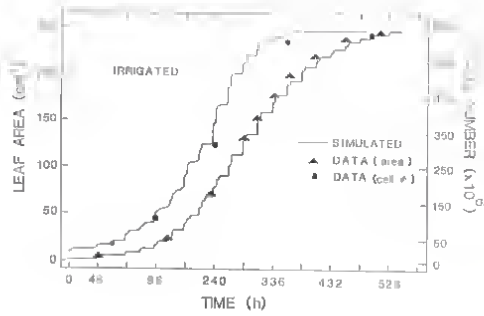

Figure 6 3 Simulations and data (mean of 4 replications) of leaf area and cell number increase during the growth of the 6th leaf.

Conclusions--Nominal Case

Many models of growth require a Gompertz, logistic or similar function to dictate a priori the shape of a growth trajectory. It is significant that it has been possible for the first time to portray accurately the growth of a leaf in terms of cell division and expansion without the use of any predetermined notion of the shape of a growth curve. Simulated and actual data are nearly identical over the entire growth period. A sigmoid shape of leaf area increase resulted from hourly calculations over a 22 day period. Furthermore early increase in both leaf area and cell number were exponential. Cessation of simulated cell division 7 to 8 days in advance of full expansion mirrored the inter-relationship between cell division and expansion in a growing leaf.

Parameter Analysis

To perform a sensitivity analysis of each parameter of the soybean leaf growth model (Fig. 6.1), all parameters were held at their nominal value(s) except the one which was varied through an appropriate range of values. Each parameter was evaluated to determine its effect on the number of cells and area of a fully expanded, well-watered leaf. Each parameter was tested utilizing three criteria to terminate leaf growth: 1) when the average cell volume was 5000 um^3 or less, 2) at 556 h or 3) at 720 h. Nominal values or equations of model parameters are listed in Table 6.1 to aid in the interpretation of the parameter analysis.

Leaf Wall Extensibility

A nominal leaf wall ext[illegible] h^{-1} h^{-1} was employed in simulations of growth of th[illegible] leaf under well watered and droughted conditions (Table 6.1). The range of leaf wall extensibility tested was 0.02 to 0.20 MPa^{-1} h^{-1}. The resultant range of cell numbers in fully expanded leaves was simulated to be 50 million to 1.4 billion (Fig. 6.4). The three criteria used to terminate leaf growth resulted in no difference in the final number of cells for leaf wall extensibility greater than 0.10 MPa^{-1} h^{-1}. Below 0.10 MPa^{-1} h^{-1}, the average cell volume increased beyond 5000 um^3 if leaf growth continued beyond 556 h.

The effect of leaf wall extensibility on final leaf area is plotted in Fig. 6.5. Increasing leaf wall extensibility beyond 0.09 MPa^{-1} h^{-1} did not influence final leaf area. Below 0.09 MPa^{-1} h^{-1}, leaf area decreased rapidly to 15 cm^2 at 0.02 MPa^{-1} h^{-1}. With the exception of 0.06 MPa^{-1} h^{-1}, none of the stopping criteria yielded significantly different final leaf areas over the range of leaf wall extensibility tested.

Leaf Wall Yield Threshold

Leaf wall yield threshold was altered through changing the equation which related leaf wall yield threshold to leaf area (eqn. 6.2). The intercept of this equation, termed the initial leaf wall yield threshold, was varied between 0 and 0.6 MPa, with a nominal value of 0.3 MPa for well-watered leaves. Final cell number increased

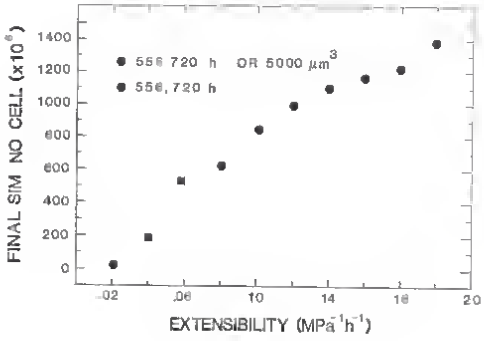

Figure 6 4 Simulated changes in the number of cells over a range of wall extensibilities and with three criteria used to stop leaf growth.

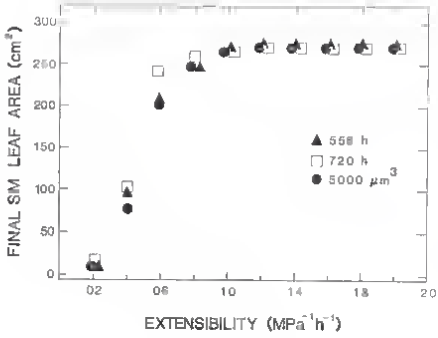

Figure 6.5 Simulated changes in the final leaf area over a range of wall extensibilities and with three criteria used to stop leaf growth

with decreasing initial leaf wall yield thresholds (Fig. 6.6). The final number of cells produced was independent of the stopping criteria for initial leaf wall yield thresholds greater than 0.3 MPa. For lower values, cell volume increased beyond 5000 μm^3 when leaves were permitted to grow beyond 556 h.

The influence of the initial wall yield threshold on final leaf area is plotted in Fig. 6.7. Final leaf areas were mostly unaffected by initial wall yield threshold less than 0.3 MPa. An increase in the initial wall yield threshold above 0.3 MPa reduced final leaf area dramatically. None of the three stopping criteria had a significant impact on the final leaf area over the range of initial wall yield threshold tested.

Cell Division Slope

The slope of the minimum volume-division function (eqn. 6.6) termed the cell division slope, specifies the rate of increase of the minimum cell volume required for cell division. From eqn. (6.6), the nominal cell division slope was 12 $\mu m^3\ h^{-1}$. The slope was varied between 3 and 20 $\mu m^3\ h^{-1}$, and the effect on the final cell number produced is presented in Fig. 6.8. Increasing the cell division slope decreased the final number of cells. Criteria used to stop leaf growth had no effect on the number of cells produced at slopes less than 12 $\mu m^3\ h^{-1}$. With an increase in the cell division slope beyond 12 $\mu m^3\ h^{-1}$, average cell volumes were greater than 5000 μm^3 when simulations extended beyond 556 h.

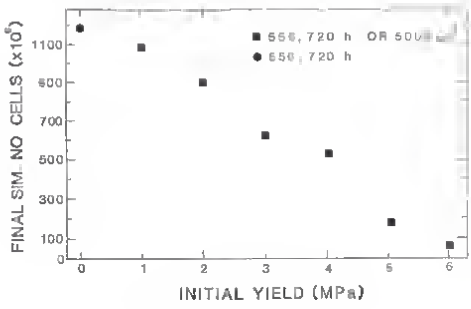

Figure 6.6 Simulated changes in the final number of cells over a range of initial wall yield thresholds and with three criteria used to stop leaf growth

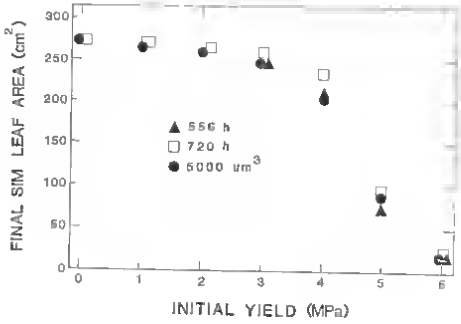

Figure 6 7 Simulated changes in the final leaf area over a range of initial wall yield thresholds and with three criteria used to stop leaf growth.

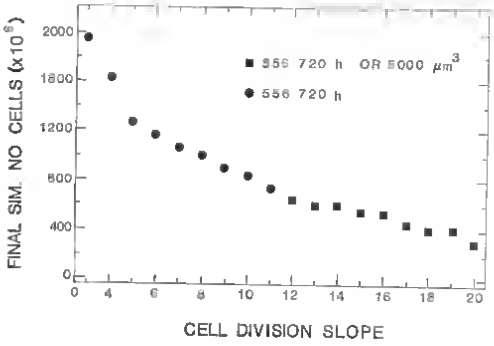

Figure 6.8 Simulated changes in the number of cells over a range of cell division slopes and with three criteria used to stop leaf growth

[illegible] $^{-1}$, did [illegible] [illegible] resulted in a [illegible] leaf area [illegible] than 130 cm [illegible] 20 um^3 h^{-1} (Fig 6 9). [illegible] af area was essentially the same whether growth was stopped at [illegible] or 720 h over the range of cell division slopes examined. For [illegible] division slopes greater than 12um^3 h^{-1}, when leaf growth was terminated by limiting average cell volume to 5000 um^3, final leaf areas were lower than when 556 or 720 h were used as stopping criteria.

Initial Cell Volume Required for Division

The intercept of the maximum volume division function (eqn 6 3) corresponds to the volume for cell division of a leaf that just emerged from the meristem. The nominal value is 800 um^3. The initial cell volume for division was varied between 400 and 1400 um^3. The final number of cells decreased asymptotically with decreasing initial cell volume required for division such that there was only a difference of 25 million cells between initial cell volumes of 800 and 1400 um^3 (Fig 6 10). For initial cell division volumes of 800 and 1400 um^3, each of the stopping criteria resulted in identical cell number at the end of leaf growth. When cell division volumes were smaller than 800 um^3 at the start of leaf growth, average final cell volume increased beyond 5000 um^3 when leaf growth continued beyond 556 h. The final leaf area was essentially constant over the range of initial cell division volumes tested (data not shown).

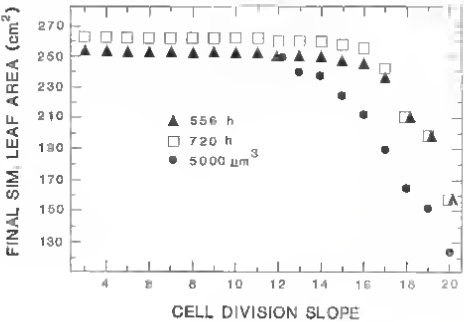

Figure 6.9 Simulated changes in the final leaf area over a range of cell division slopes and with three criteria used to stop leaf growth.

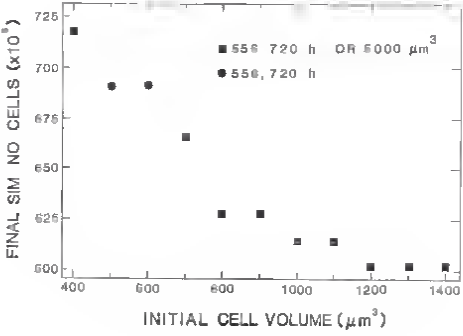

Figure 6.10 Simulated changes in the number of cells over a range of initial cell volumes and with three criteria used to stop leaf growth

[illegible]y of Parameters [illegible] Leaf Growth

Sensa[illegible]sis were performed on the following four parameters: 1) cell extensibility, 2) initial wall yield threshold, 3) cell division slope and, 4) the initial cell volume. Slight increases from the nominal value of the initial wall yield threshold (0.30 MPa), or the cell division slope ($12 cm^3 h^{-1}$) resulted in large decreases in the final leaf area. Similarly, a slight decrease of the wall extensibility from its nominal value of 0.06 $MPa^{-1} h^{-1}$, resulted in a sharp drop in the fully expanded leaf area. This indicates that these variables may control the growth of soybean leaves with only small shifts in value from their normal levels. Van Volkenburgh and Cleland (1986) ascribed leaf growth regulation to the wall extensibility in their study of bean (Phaseolus vulgaris) leaf expansion. Cosgrove (1981) also determined through simulation that wall yield threshold and wall extensibility could play significant roles in the control of individual cell expansion.

Significantly, the area of fully expanded leaves was not increased by variation of any of the four parameters examined. Changes in these parameters expected to result in an increase in the fully expanded leaf area resulted instead in increases only in the number of cells.

Sensitivity to Turgor Potential

Simulations were conducted to examine the impact of various levels of leaf turgor potential during leaf growth on the number of cells and area produced in a fully expanded soybean leaf. Leaf turgor potential throughout a day was described by eqn (6.4) for a well-watered leaf. To obtain a range of leaf turgor potentials, this equation was modified by shifting the average +0.25 MPa in units of 0.05 MPa. Leaf turgor potential was then averaged over a 24 h period to obtain a single integrated turgor value called the average daily turgor. The average daily turgor potential is 0.57 MPa for eqn (6.4) (irrigated) and 0.32 MPa for its droughted counterpart (eqn. 6.5) given in Table 6.1.

The influence of different average daily values of leaf turgor potential throughout the development of a leaf on its final number of leaf cells is plotted in Fig. 6.11. The number of cells in a fully expanded leaf increased with increasing average daily turgor. The average daily turgor of plants droughted for 8 days (0.32 MPa) resulted in less than 100 million cells produced, while turgor observed in plants irrigated every 2 days (0.57 MPa) resulted in 625 million cells at the end of leaf growth. None of the criteria used to stop leaf growth resulted in different numbers of cells.

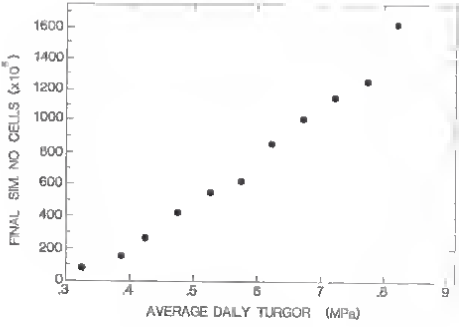

Figure 6 11 Simulation of the effects of the average daily turgor on the number of cells in a mature leaf and with three criteria used to stop leaf growth.

Average daily turgor is plotted with respect to the area of a fully expanded leaf in Fig 6.12. Final leaf area was less than 50 cm^2 at the lowest average daily turgor and approached a maximum leaf area of 400 cm^2 at average daily turgors of 0.82 MPa. All stopping criteria resulted in similar final leaf areas for the range of turgor examined.

Simulations of leaf growth in response to drought induced changes in turgor and wall yield threshold. Simulations of leaf growth, utilizing values of leaf turgor potential and wall yield threshold measured during drought, were performed to examine model performance and to assist in understanding the characteristic responses of leaves to drought observed in the previous chapters. In these simulations, drought was imposed throughout the entire growth period of a leaf. The values of parameters used during drought are listed in Table 6.1

Simulated number of cells with time is presented for a droughted and, for comparison, a well-watered leaf (Fig. 6.13). The production of cells during the first 6 days of growth of the droughted leaf lagged considerably behind that of the well-watered leaf. Also, the time to onset of the linear increase in cell number was delayed for several days. Interestingly, cell division of droughted leaves continued four days beyond the time when cell division ceased in the well watered leaf. Overall, cell production in the droughted leaf was 28% less than in the well-watered leaf by the end of growth.

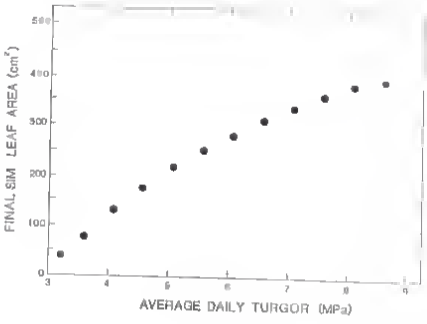

Figure 6.12 Simulation of the affects of the average daily turgor on the final leaf area.

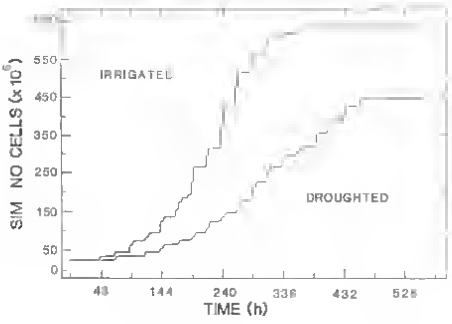

Figure 6 13 Simulation of changes in the number of cells with time during well watered and droughted conditions

[illegible] Fig. 6.[illegible]
[illegible]s and well-watered leaves. During the first 4 days of growth, comparison of the droughted and well-watered leaves indicates that area changes in this interval are not particularly sensitive to low turgor and higher wall yield thresholds. Leaf growth during this interval may depend on the same physiological processes that occur in very small leaves within the meristem. If so, then the simulations support observations made in Chapter II that leaf initiation is not as sensitive to drought as is leaf area expansion. However, after the 4th day, the simulated increase in area of the droughted leaf lagged considerably behind that of the well watered leaf. The final area the droughted leaf attained was approximately 30% lower than the well-watered leaf.

Leaf relative growth rate. Simulated relative growth rate for a droughted leaf, when plotted as a function of normalized leaf area, was marked by daily fluctuations throughout the growth of the leaf (Fig. 6.15). Similar plots were obtained for a well-watered case (data not shown), the only difference being that the highest value of relative growth rate was 0.01 s^{-2} m^{2} h^{-1} greater in the well-watered than in the droughted leaf. Daily fluctuations in the relative growth rate may be described as parabolas which open downward and whose vertices decrease as the leaf ages. Each base of the parabolas rests on the abscissa which indicates that at one time per day (at approximately 1400) the relative growth rate was zero. Each vertex corresponds to 0200 when leaf turgor is at its maximum value as is growth

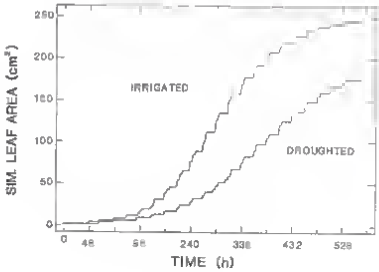

Figure C_14 Simulation of changes in the area of a leaf with time during well watered and droughted conditions.

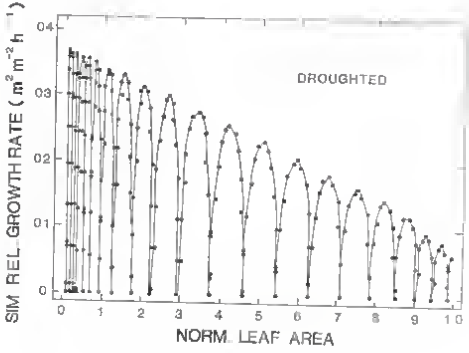

Figure 6.15 Simulated leaf relative growth rates versus normalized leaf area for a droughted leaf.

The simulation results are consistent with relative growth rate data from July, 1984 (Table 5.3), when a significant drop was found in the relative growth rate for data collected between 1200 and 1800 relative to the other periods. Relative growth rate was also higher in the morning and evening when turgor is expected to rise. In a study of the diurnal growth of corn under field conditions (Zea mays), Thut and Loomis (1944) indicated that corn leaf growth occurred during intervals when turgor would be expected to be lowest. However, the source of growth in corn may be sufficiently removed from environmental fluctuations to permit growth during times of high solar radiation and temperature (Michelena and Boyer, 1982).

For the study of soybean leaf growth (July, 1984), weather conditions during the first several days were sunny and hot (Fig. 6.16). Growth of the 8th leaf during this time was expressed several ways in Table 6.2: as an absolute area increase between 0600 and 1800 (day) and between 1800 and 0600 (night) and, as a relative increase in area (day/night). From 15 - 17 July (days 197 - 199), air temperatures and solar radiation were high. During that time, leaf turgor would be expected to be low. From Table 6.2, absolute leaf growth was lower during the day than at night and the ratio of day to night growth was approximately .33. Over the following two days, air temperature and solar radiation moderated while growth during the day began to increase relative to growth during the night. This is consistent with the effect of drought on a reduction in the greatest magnitude of growth during the day as indicated by Fig. 6.15.

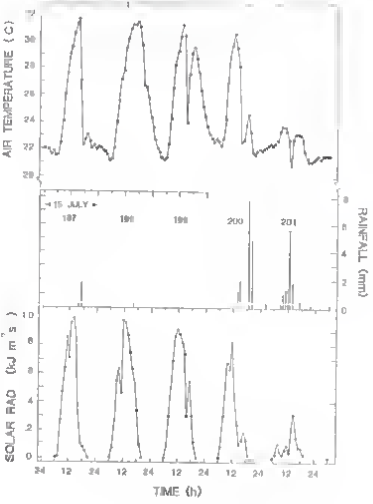

Figure 6.16 Solar radiation, temperature and rainfall for the period of 15 through 19 July, 1984.

Increase ar … as an absolut… during … 0600 – 1800 and night (180… 0600) and a… io of night and day growth…

Day of year	Day (0600 – 1800)	Night (1800 – 0605)	Day/Night
	leaf area increase (cm^2)		
197 – 198		24.3	-
198 – 199	14.3	40.3	0.35
199 – 200	10.3	33.5	0.32
200 – 201	17.9	10.0	1.79
201 – 202	11.1	14.5	0.77

Effects of leaf development during drought. The effect of drought on the growth of leaves is complicated by the fact that leaves on a single plant are in various stages of development. The importance of this condition was outlined in Chapter III during the discussion of the differential sensitivity of small and large leaves to drought. To investigate the influence of drought on the development of leaves, a simulated drought was imposed upon leaves of different sizes for a 5-day period (see Chapter III). Drought was simulated by reductions in leaf turgor and leaf wall yield threshold as mentioned earlier (Table 6.1).

A reduction in the final simulated leaf area and number of cells relative to the irrigated case is presented in Fig. 6.17 for leaves droughted at different initial leaf areas. This may be compared with data collected in 1984 (Table 3.2) which is presented in graphical form as Fig. 6.18. Simulated leaf area was most influenced when leaves were initially less than 40 cm^2, although there was considerable reduction for initial leaf area up to 118 cm^2. When leaves had completed approximately 70% of their growth (196 cm^2), cell division was not reduced by a 5-day drought, but leaf area continued to decline by 10%. The final number of cells was reduced mostly for small leaves, i.e. those having initial areas less than 40 cm^2.

Simulations of the influence of drought on leaves of differing developmental stages were reasonable depictions of data collected in 1984. Both data and simulations show that smaller leaves were more sensitive to drought as expressed by reductions in final leaf area and cell number. Simulations would have been more realistic if leaf cell

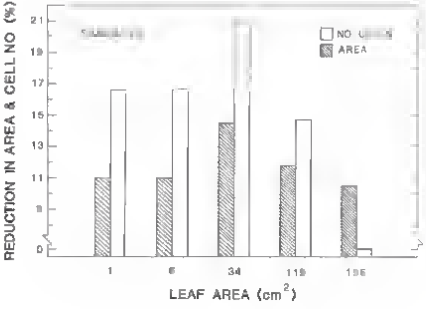

Figure 6.17 Simulated reductions in the final area and number of cells from leaves droughted for 5 days at different stages of development

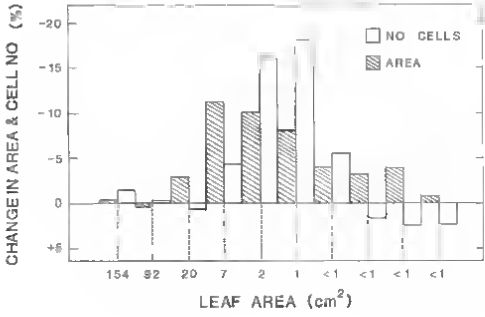

Figure 6.18 Changes in the final area and number of cells from leaves droughted for 5 days at different stages of development

division occurs [illegible] (Fig 6.13). Continued cell division [illegible] the development of droughted leaves may be an adaptation to drought which would enable leaves to expand cells not normally available for growth

Discussion

Relationship between Cell Division and Expansion

The concept that cells must increase in volume before undergoing division was embodied in the minimum volume division function (eqn 6.3). It is apparent that cells increase in volume before they divide, yet considerable effort has been paid to the identification of when each process occurs (Milthorpe, 1959; Sunderland, 1960; Dale, 1964). Clearly both processes occur simultaneously during early leaf growth.

This relationship may effectively regulate cell division. When cells cannot attain the required volume, cell division stops until cell volumes have increased sufficiently. This may explain the cyclical waves of cell division and expansion noted in the root cells of onion (Allium cepa) by many early botanists (Kellicot, 1904; Priesner, 1920). In those studies, cell division and expansion were mutually exclusive. This is to be expected in tissues having narrow ranges of cell type and volume. In a leaf, the situation is compounded by the presence of epidermal, vascular, parenchymal and mesophyl cells, all with varying sizes and turgors (Zimmermann et al., 1980). Some cells have sufficient volume to continue division while others do not.

Leaves contain cells having a broad range of volumes and their potentials. Cell types having small volumes would be the first to cease division since expansion may not keep pace with the minimum volume required for division. As the demand for greater volume for division outstrips the cells ability to expand or the environment limits the amount of turgor available to drive expansion (i.e. through drought), cell division stops. Based on a cell division model, Dale (1970) predicted that 20% of the cells at the end of division in a bean leaf should maintain the capacity for cell division. This may correspond to the continued capacity of palisade cells in soybean to divide late in the development of a leaf (Lugg and Sinclair, 1980).

Implicit in the minimum volume function is the notion that average cell volume increases linearly with time. This is supported by results from Dale (1964), who plotted estimated mean cell volume versus time for bean leaves. When linear regression of his data was calculated, an $r^2 = .94$ resulted. This implies a close coupling between all aspects of leaf development including cell morphogenesis, cell expansion, and cell division.

Conclusions of Sensitivity Analysis of Model Parameters and Turgor Potential on Leaf Growth

It is significant that of the variables used in the equations to describe leaf growth, those which directly relate to physiological processes of the cell wall (leaf wall yield threshold and the leaf cell wall extensibility) do not increase the area of a leaf when their values are changed in a direction expected to result in increased growth. These responses, taken together with the increase in leaf

area and cell number o[illegible] primarily [illegible] that increases in orde[illegible]ize may only be ac[illegible] by increasing turgor and not through alteration of wall metabolism alone

Parameters of the cell wall expansion equation appear to control the final size of the leaf regardless of the propensity of the cells for division. This fact, taken with the sensitivity of leaf area to turgor implies that leaf cell turgor is the most critical aspect of leaf area production. Historically, the association between leaf turgor and growth is long and debate filled. Leaf turgor measured in three environments showed a positive, linear relationship to leaf lamina expansion rate (Hunce, 1977). Leaf expansion also exhibited a linear relationship to leaf turgor in field grown, droughted and irrigated soybeans (Masri, 1986). Evidence contrary to any link between turgor and growth was reported by Michelena and Boyer (1983), who measured constant turgor in the growing region of corn despite variable growth rates. Likewise, with field grown soybean, instantaneous measures of leaf extension rates showed extreme dependence on environmental stimuli but showed minor fluctuations with leaf turgor as measured with a pressure chamber (Wenkert et al., 1979).

Cell turgor is an absolute requirement for cell expansion because there must be an internal pressure to push against the cell wall to initiate cell wall expansion. The variable results of investigations of leaf turgor and growth may be due to the measurement techniques used to measure turgor and, possibly, due to the inability to measure turgor on a real time basis in all of the cells of the leaf. This

[illegible] requirement is based upon the possibility that the leaf epidermis or some other tissue structure restricts lamina expansion despite turgors favorable for growth in cells located in the mesophyl

Conclusions

A mechanistic model of soybean leaf growth was presented which incorporated in a realistic way, the division of cells and their expansion. The main feature of the model is its requirement that cells expand at a rate limited by the wall relaxation equation and to divide only when a time-dependent cell volume was attained.

Sensitivity analysis indicated that only increases in the turgor potential could effectively increase the leaf area. Small decreases in variables which relate to cell wall extension resulted in large decreases in the final leaf area attained. The model was successful in portraying growth of the 6th leaf from a soybean plant during well-watered conditions. The model also performed well in predicting the nature and extent of drought induced growth reductions.

CHAPTER VII
CONCLUSION

The goal of this chapter is to summarize and put into perspective the work of the preceding five chapters as well as to suggest future work and to identify unresolved issues.

The goals of the research in this work were to first, quantify the alteration of leaf growth and development in response to drought. The second goal was then to develop and to verify a unified theory of leaf growth which would account for the characteristics of leaf growth during drought.

In Chapter II, the effect of drought on leaf emergence and leaf area expansion was investigated. The leaf area was reported to be linearly related to the plastochron index in a droughted crop of 'Bloxi' soybeans once 5 to 6 leaves had formed. Despite an earlier report (Vandeleur et al., 1982) that changes in the plastochron indices were sensitive indicators of drought stress, leaf area expansion was more severely reduced by drought.

In Chapter III, attention focused on leaf area expansion and the possible factors responsible for the characteristic reductions in the final area of leaves from droughted plants as well as the sensitivity of small leaves to drought. An hypothesis was proposed which stated that these characteristic decreases in the growth and development of leaves were dictated by the developmental stage of the leaf at the time of drought. Leaf area and cell number were counted in leaves

drought [illegible] different phases of their development. The results support the hypothesis and suggest that characteristic growth reductions were due to differential sensitivities which exist between cell division and cell expansion.

In Chapter IV, the first stage of a unified theory of leaf growth was constructed which incorporated the response of leaf growth to drought. Because leaf growth is dependent in large part upon cell expansion, an analogue of the cell wall relaxation equation (eqn. 7.1) was proposed as a means to describe the bulk expansion of cells within a leaf.

$$(1/A)dA/dt = \phi(P - Y) \qquad (7.1)$$

Two terms in this equation, the leaf turgor potential (P) and the wall yield threshold (Y) were measured under field conditions. After theoretical and experimental verification of the techniques used to obtain and interpret the leaf wall yield threshold, data from three years of experimentation were presented. Leaf turgor potential decreased in droughted plants while the wall yield threshold increased in small, droughted leaves. The leaf wall yield threshold also was found to vary linearly as a function of normalized leaf area for both well watered and droughted leaves.

The remaining terms of the modified cell wall relaxation equation (eqn. 7.1), the leaf relative growth rate ($(1/A)dA/dt$) and the leaf wall extensibility (ϕ) were examined in Chapter V. Relative growth rate was measured in the leaves of well-watered and droughted field-grown soybean plants. These data, when expressed as a function of normalized area, indicated that leaves which had completed 20% of

their growth expanded at a rate which was [illegible] their water status and linearly related to their size. The wall relaxation equation was then algebraically manipulated to solve explicitly for the wall extensibility

$$\phi = (1/A)dA/dt \;/\; (P - Y) \qquad (7.2)$$

Wall extensibility was determined from equation (7.2) and it is expressed in a plot (Fig. 5.3) to indicate its dependence upon normalized leaf area and leaf turgor during growth. Wall extensibility was essentially the same for droughted and well-watered leaves and was similar to values reported by Cosgrove (1981 and 1985). However, due to the range in values, it was not possible to determine if wall extensibility played any role in controlling growth responses during drought.

In Chapter VI, information derived from the wall relaxation equation (eqn. 7.1) and from leaf cell population data presented in Chapter III, was synthesized into a leaf growth model. The model linked cell division and cell expansion events by means of an equation called the minimum volume division function (eqn. 7.3):

$$S(t) = 12*t + 800 \qquad (7.3)$$

where t is time (h). This equation required cells to attain a time dependent, minimum volume before they could divide. Therefore, cell division could be effectively regulated by cell expansion.

The leaf growth model accurately predicted leaf blade expansion as well as the increase in the number of cells in a leaf with time. The model was then used to assess the impact of various parameters of the wall relaxation equation and the minimum volume division function

on leaf growth. Only an increase in turgor potential was capable of increasing leaf area. Simulations performed with the model successfully predicted the growth reduction noted in small leaves and related the reduction in final leaf area and cell numbers to the stage of leaf development at the time of drought.

Conclusions

Recently it was proposed (Sinclair and Ludlow, 1986) that the thermodynamic viewpoint has 'not led to any profound insights about physiological response to water deficits'. Cell turgor potential has occupied a central role in both the division and expansion of cells in the model developed in this work. The role of turgor potential in the success of the leaf growth model has reaffirmed the importance of thermodynamically based concepts of the energy status of water in living systems. Turgor potential has been shown to be crucial to the physiological response of leaves to water deficits.

The second basic conclusion of this work is that leaf growth is definable through description of its underlying processes (i.e. that the whole is but a sum of its parts). Through the success of the leaf growth model and the concepts therein, it has been possible to employ the processes of cell division and cell expansion to describe changes at the tissue and organ level.

The third conclusion is that once cell division is separated from associated processes which occur concurrent to division (protein and cellulose synthesis and membrane construction, etc), the division of cells per se is not particularly sensitive to drought since the

minimum volumetric diffusion resistance (eqn. 7.3) sufficed for well-watered and droughted conditions.

On a more practical level, three characteristic growth responses to drought have been identified:

i) slight delay in the formation of leaves within the meristematic region,

2) high sensitivity of leaf size formation in small leaves,

3) end reduction of final leaf area depends upon the stage of growth at the time of drought

No conclusions can be drawn about the mechanisms responsible for the maintenance of leaf formation in the meristem since the leaf growth model only considered changes that occurred once the leaf emerged. There is evidence that all leaves are formed within the first 35 days of growth (Johansen et al., 1980). This would allow effective avoidance of drought by forming these structures before competition for water with adjacent plants occurs.

Both the second and third characteristic growth responses are functions of the effect of drought on cell expansion and the requirement that cells expand before dividing. In small leaves, drought could reduce the expansion and division of cells more acutely than in larger leaves since larger leaves have a lower proportion of cells in a state of active cell division. The case for reductions of final leaf size relative to its size at the time of drought follows from the temporal sequence of cell division and expansion in large and small leaves. During drought, young leaves suffer proportionally greater growth reduction than more mature leaves since the effective

[illegible] stage of [illegible] also retarded expansion volume is insufficient [illegible] and cell expansion likewise is reduced. Drought midway through the growth of larger leaves has less effect on cell production because fewer cells are being produced. Upon rewetering, there are almost as many cells to expand relative to the control leaves.

Unresolved Issues

There has been no mention of the role temperature plays in the division and expansion of cells. The minimum-volume division function (eqn. 7.3), which was originally defined in terms of volume changes per unit time, may be more related to temperature than time. An analogy may be drawn with the common observation of linearity in the production of leaves over time. This response actually has been shown to consist of a response to a steady temperature environment (Hesketh et al., 1973). The relationship between cell division and expansion clearly needs to be defined better in regard to temperature variations.

Similarly, a final goal of many individuals would be to connect growth processes as described in this work, to photosynthesis and respiration. Photosynthesis is required to produce the solutes needed to construct the cell machinery and to provide the energy for respiration. How these processes are tied in with leaf development is not clear.

The leaf growth model has been singularly successful with soybean in Gainesville, Florida. The acceptance of the ideas in the model will only come to pass if tested in different environments and with different crops. Similarly, the prediction of leaf area increase by increasing the turgor potential should be examined, possibly with high frequency irrigation studies. Water may be the vital chemical missing from the shelves of chemical companies looking for ways to increase crop yields.

APPENDIX A
THEORETICAL AND EXPERIMENTAL VALIDATION OF WALL YIELD THRESHOLD MEASUREMENT IN VAPOR PRESSURE PSYCHROMETERS

The use of vapor pressure psychrometry to measure the wall yield threshold of plant tissue has been proposed only recently (Cosgrove et al. 1984). The measurement of wall yield threshold may depend upon the geometry of a psychrometer assembly, as it pertains to heat and water vapor transfer, as well as the physiological characteristics of the tissue under investigation. Therefore, theoretical estimates of heat and water vapor transport in the psychrometer assembly were examined and where possible, experiments were performed to validate those results. Physiological traits of soybean leaf tissue which may influence the measurement of wall yield threshold also were examined theoretically and, where possible, experimentally evaluated.

Successful measurement of yield threshold in a psychrometer should require: 1) a steady osmotic potential during wall relaxation to ensure that changes in the pressure potential not associated with relaxation of cell walls not occur, and 2) sufficient time to complete wall relaxation and associated thermal and vapor equilibration. Each of these requirements will be examined as they pertain to either the physiological status of leaf tissue or the heat and vapor transport characteristics of a psychrometer assembly.

The results indicate that heat transport [illegible] of psychrometer assembly equilibrate very rapidly with the water bath used to maintain thermal stability. Water vapor transport to the sensing junction in the psychrometer chamber is dependent upon the leaf cuticular conductance. Experimental and theoretical results indicate that water vapor requires approximately 2-5 h to equilibrate throughout a soybean leaf tissue - psychrometer system. Soybean leaf tissue displayed little physiological activity during equilibration which would interfere with measurement of the wall yield threshold. Water potential is measurable in soybean leaf tissue within 3 h after being placed into a psychrometer assembly. The wall yield threshold may be measured after a 3 h equilibration period.

Tissue Properties

Theoretical Examination

Estimation of change in osmotic potential associated with respiratory consumption of substrate. Given the respiration rate of young leaf tissue, it is possible to estimate the amount of sucrose it will consume and then to determine if that amount of sucrose is an osmotically significant proportion of the total.

Assume that a young, rapidly respiring leaf produces 15 mg CO_2 100 cm^2 h^{-1}. Also since:

1 mmole CO_2 = 44 mg CO_2,

1 mmole hexose = 6 mmole CO_2,

1 mmole hexose = 0.5 mmole sucrose,

sample leaf area = 0.78 cm^2,

equilibration time = 4 h,

then 8.92×10^{-4} moles of sucrose are consumed per leaf sample per equilibration period.

To calculate the corresponding drop in osmotic potential, the osmotically active volume of the leaf sample is estimated as follows:

Assume: leaf thickness = 0.015 cm,

leaf area = 0.78 cm^2,

airspace in leaf = 20%,

osmotically active water = 85% of total cell volume,

density of water = 1 g ml^{-1}.

The osmotically active mass per leaf sample is calculated to be 7.96×10^{-5} kg of water. The molality of sucrose lost to respiration is the moles of sucrose per kg of osmotically active water:

8.92×10^{-7} moles sucrose / 7.96×10^{-5} kg water

or 0.012 molal sucrose. The corresponding change in water potential is then calculated with the Van't Hoff relation to be 0.028 MPa. This is the theoretical drop in the osmotic potential of leaf tissue equilibrating after 4 h in a psychrometer chamber held at 30°C. It was not necessary to consider changes in osmotic potential due to hydrolysis of starch since rapidly growing cells contain little if any starch granules.

Experimental Validation

Materials and methods. Actively growing leaf tissue from leaves recently emerged from the meristem were sampled at 1100 EST. Thirty six samples, each consisting of three leaf punches of 6 mm diameter,

were pl[illegible] [illegible] [illegible] [illegible] and immersed in [illegible] water bath. Six more leaf samples were gathered, immediately pl[illegible] into a brass chamber, sealed and placed into a freezer held at (15°C). Freezing caused the cell membranes to rupture. At hourly intervals, water potentials of the samples immersed in the water bath were read with a dew-point microvoltmeter and recorded on a strip chart recorder. Six chambers were then taken from the water bath, and the chambers containing tissue were removed from the psychrometer, sealed with a brass screw cap and then placed into a freezer. This procedure was repeated every hour until all samples had been measured for their total water potentials, removed from the bath and placed into a freezer.

After the leaf tissue in the chambers had been in the freezer for 2 days, they were removed and allowed to sit on a counter until they equilibrated with ambient air temperatures. The sample chambers were then reattached to psychrometer assemblies and placed into a 30°C water bath for 4 h. Osmotic and total water potentials were then measured as described earlier.

Results and Discussion. The osmotic potentials of samples measured for six consecutive hours were 1.03, 0.97, 0.98, 1.01, 0.99, 0.96 MPa. The total water potential measured at the end of the 4th, 5th and 6th hour expressed as the mean of the three hourly values (± stand error) was 0.72 (± 0.04) MPa.

The lack of statistically significant differences among the osmotic potentials of samples removed from the water bath at hourly intervals up to 6 h indicates that osmotic potential was stable

throughout equilibration [illegible] differences a[illegible] the last three water potential [illegible]s indicate that this value was also stable after 4 h of equilibration. Prior to that time, total water potentials declined from a maximum value measured at 30 min.

It was concluded that the osmotic potential in rapidly growing leaf tissue is not significantly changed during 6 h equilibration in a 30°C water bath. This supported evidence from the theoretical treatment dealing with respiratory consumption of substrate that the osmotic potential was stable throughout equilibration.

Heat Transport Theory

The initial requirement of any psychrometric determination of water potential is the achievement of a spatially uniform, time invariant temperature amongst all parts of the plant tissue, the psychrometer assembly (psychrometer plus sample chamber) and the air and water vapor contained within. Typically, the initial temperature of the plant tissue and psychrometer assembly are different than that of the water bath which is used as means to describe a uniform temperature. Therefore, as a first step toward determining a minimum time lag before water potential measurement, the dynamics of heat transport of the water bath, psychrometer chamber and plant tissue were examined.

A general model of heat transport was developed to study the problem of achieving temperature equilibration in a psychrometer system. Due to the complex geometry and nonhomogeneous nature of the psychrometer assembly and plant tissue, an electric analog model was

utilized to describe thermal energy tra[illegible] regions of the system. Each region is described by a thermal resistance and thermal capacitance. The product of the resistance and capacitance for each region yields that region's thermal time constant. Forced convection, natural convection and/or conductive diffusion models were used as appropriate across regions or at interfaces to calculate each resistance term. The capacitance term for each region is defined as the product of its mass and heat capacity. The four interfaces examined were between the: 1) water bath and outer chamber walls, 2) inner chamber walls and the internal air space, 3) inner chamber walls and plant tissue and 4) plant tissue and the internal air space.

The first consideration in the determination of heat transfer variables at the water-chamber wall interface is the magnitude of forced versus natural convection. The speed at which water flows over the chamber walls will to a large extent determine which type of heat transfer predominates. General estimates of the magnitudes of these two effects may be calculated by a comparison of the Reynolds (Re) and Grashoff (Gr) numbers in the following fashion:

if $Re^2 >> Gr$ then forced convection predominates, otherwise

if $Gr >> Re^2$ then natural convection predominates (Karlekar and Desmond, 1977). For the water bath:

$$Re^2 = (V\,d/\nu)^2 = 9.17 \times 10^4 \qquad (A\text{-}1)$$

and

$$Gr = (g\,B\,T\,l^3)\nu^{-2} = 6.57 \times 10^4 \qquad (A\text{-}2)$$

where the value and definition for each term are given in Table A-1. Since Re^2 and Gr are of the same order of magnitude, free and forced

[illegible] models [illegible] both expected [illegible] The [illegible] convection model was chosen as the [illegible] estimate heat [illegible] ter at this interface.

As mentioned earlier, a resistance and capacitance term are calculated at each interface. The resistance term for natural convection is inversely proportional to the heat transfer coefficient h ($W\ m^{-2}\ C^{-1}$), times the area of the water-chamber wall contact (m^2) (eqn A.3).

$$R_{conv} = h^{-1} A^{-1} \qquad (A.3)$$

The heat transfer coefficient is a measure of the effectiveness of heat transport per unit area per unit degree and is a function of the thermal properties of water and the cross sectional area through which heat transfer occurs. It is given by the formula

$$h = (Nu\ K)/l \qquad (A.4)$$

where Nu is the Nusselt number, K ($W\ m^{-1}\ K^{-1}$), is the thermal conductivity and l is the length (m) of the chamber wall. For a vertical cylinder:

$$Nu = 0.59\ (Pr\ Gr)^{.25} \qquad (A.5)$$

(Karlekar and Desmond, 1977), where Pr is the Prandtl number. The later number is a dimensionless property which relates the diffusion of momentum to the rate of diffusion of heat through a fluid. The Nusselt number at this interface is 15.61, so that h, the heat transfer coefficient becomes 466 $W\ m^{-2}\ K^{-1}$ and the resistance is 2.89 $K\ W^{-1}$. The mass of the brass chamber times the heat capacity and is equal to 19.25 $J\ K^{-1}$. The time constant for transport of heat from

the water bath to the inner chamber wall is equal to the resistance to heat transfer multiplied by the product of the heat capacity and chamber mass. This time constant is approximately 56 s.

The chamber wall-air interface represents a second boundary which must be assessed for heat transport. The chamber wall air interface refers to contact between the inner chamber wall and the small volume of air within the chamber. Natural convective forces are inhibited by very low Grashoff levels within this small sealed volume. Consequently, conduction will be the limiting process through this nearly stationary gas.

The resistance due to heat conduction within a vertical cylinder is given by Karlekar and Desmond (1977) as:

$$R_{cond} = \ln 2/(2 K_{air} h) \qquad (A 6)$$

which yields $R_{air} = 0.68 K W^{-1}$. The time constant (product of the above resistance and the heat capacity of the air) for heat transport through this interface is less than 1 s. This low time constant is produced primarily by the low specific heat capacity of the air.

A third boundary is the chamber wall plant tissue interface. The psychrometer chamber wall makes contact with a portion of the plant tissue. Simplification of the geometry of the contact between the chamber walls and the plant tissue was required. For modelling purposes, it was assumed that the plant tissue was a circular solid, having a thickness of 2 mm and positioned at the base of the psychrometer chamber. This geometry approximates most measurements with leaves and other plant organs which are punched or cut to the internal dimensions of the chamber. The thermal properties of plant

[illegible] were assumed to [illegible] disc of water [illegible] for comparison [illegible] a disc of air [illegible] conditions

By analogy to the circular [illegible] in a psychrometer chamber, a conduction equation [illegible] to the slab was used to estimate the resistance parameter and is given as:

$$R_{cond} = (thickness/2)/(Area\ K) \qquad (A\text{-}7)$$

(Karlekar and Desmond, 1977), where the values for the thermal conductivity, K, and the area are given in Table A-1. The resistance due to conductive diffusion is 13 °K W^{-1} and the thermal capacitance based on the mass for water is 2.1 J°K^{-1}. The product of the resistance due to conductive diffusion and the thermal capacitance gives a time constant of 27 s. If the plant tissue is assumed to consist mostly of air, then R_{cond} = 250 °K W^{-1}, C_{air} = 2.4 X 10^{-4} J°K^{-1}, and the time constant is 6.96 X 10^{-2} s. Clearly the assumption that the plant tissue is composed of water yields the more conservative estimate of the heat transport rate.

A fourth boundary is the plant tissue-air interface. Similar to the previous interface, the plant tissue-air interface was modeled as a horizontal mass of air underlain by warmer plant tissue (as a pool of water). Equation (A-7) was used to calculate the resistance using the thermal properties of air and the geometries given in Table A-1. The following intermediate values were obtained: Gr = 8.5, Nu = 0.15(Pr Gr)$^{.333}$ = 0.277 (Karlekar and Desmond, 1977) and h = 4.0 W m^{-2} °K^{-1}. These values may be used to calculate the resistance when natural convective forces predominate via eqn. (A-3) to yield R_{conv} = 3,500 °K W^{-1}. The resistance due to conduction through a horizontal

[illegible] (7.20) where R_{cond} = 461 [illegible] capacitance of the air contained within the chamber, 1.49 X 10[illegible] W^{-1}, dominates the time constant calculation. The time constants for the conduction and natural convection heat transport at the plant tissue air interface are 0.72 and 5.23 s, respectively.

Conclusions

The heat flux is dominated by the interface having the longest time constant. The time constant of the water bath chamber interface was approximately 56 s. If 5 time constants represents complete equilibration, then the psychrometer and plant tissue within should be equilibrated within 4.7 min.

Heat Transport--Experimental

Heat transport in the water bath psychrometer assembly system was studied by measuring temperature changes with a copper constantan junction located within the body of the psychrometer. This permitted an approximate measurement of the time constant for heat transport through the water bath-inner chamber wall interface since the location of the sensing junction was presumed to have been located several millimeters internal to the inner chamber wall. Temperature changes were not monitored at the other interfaces since the time constant of the water bath-inner chamber wall region dominated the transport of heat throughout the system.

Materials and Methods

Twenty psychrometer chambers were filled with a single 9 mm diameter filter paper disc which had been soaked in deionized water. Initial chamber temperature was 25°C. The chambers were attached to psychrometers and individually placed into a 30°C water bath at 5 min intervals. Temperatures in the psychrometers were continuously monitored in the temperature mode of a Wescor Dew-point Microvoltmeter and recorded by a strip chart recorder. Time constants for each psychrometer were computed from plots of temperature versus time.

Results and Discussion

The mean time constant (± stand. error) for temperature equilibration between the water bath and the temperature sensing junction was 36 (± 4) s. This time constant is similar to the one obtained from the theoretical treatment of heat transport through the water bath psychrometer-plant tissue system (56 s). This indicates that the psychrometer is capable of achieving rapid thermal equilibration necessary for water potential or wall yield threshold measurement.

Vapor Transport Theory

The basis of psychrometric measurements of water potential is the attainment of thermodynamic equilibrium between the water located within the cells and the vapor in the volume of air surrounding the psychrometer measurement junction. The time required for equilibrium

[illegible] is dependent upon the route of [illegible] transport through the [illegible] to the tissue surface and the distance the vapor must then travel before reaching the sensing junction (Boyer and Knipling, 1965). The latter transport process was examined by Lambert and van Schilfgaarde (1965), who employed a one dimensional diffusion model of the following form:

$$U_t = D\,U_{xx} \qquad (A.8)$$

where U is the vapor pressure (Pa), t is time (s), x is distance (cm) and D is the diffusion coefficient ($m^2 s^{-1}$). The initial vapor pressure at the surface of the tissue U' was assumed to be in equilibrium with the water within the tissue and, at the opposite end of the chamber the vapor flux U_x, was set equal to zero for all time. The mathematical representation of these boundary conditions can be stated as:

$$U_x(L,t) = 0 \qquad (A.9)$$

$$U(0,t) = U' \qquad (A.10)$$

where L is the length of the psychrometer chamber. For a length of 2 cm and an initial vapor pressure equivalent to a relative humidity of 50%, essentially saturated conditions were obtained at L within 20 s.

Model of Vapor Transmission--New Boundary Conditions

The initial point of departure of water vapor in the psychrometer assembly does not occur at the surface of leaf tissue as had been modeled by Lambert and van Schilfgaarde (1965). Instead, water vapor must first diffuse through a barrier consisting of the cuticle and stomata. Having now defined the barrier to diffusion at the surface

[illegible] formulate the boundary condition [illegible] following boundary condition is obtained

$$[illegible] = K^*(U(0,t) - U_s) \qquad (A\ 11$$

where [illegible] is the epidermal conductance to water vapor flux, $U(0,t)$ [illegible] the absolute humidity just above the plant tissue for all time and U_s is the constant, saturated absolute humidity at some point within the leaf tissue. This later value may not be constant if, as has been shown by Bennett and Cortes (1965), the mass of vapor is insufficient to saturate the walls of the psychrometer. This problem is generally avoided through the use of sufficient plant tissue. Equation (A-8) with boundary conditions given by eqns. (A-9) and (A 10) was solved by Peck (1965) from examples provided by Carslaw and Jaeger (1959). When K, the epidermal conductance, ranged from 0.01 m s^{-1} to 0.0001 m s^{-1} (Sinclair and Ludlow, 1986), the equilibration period ranged from approximately 5 to 50 min.

Vapor Transport--Experimental Evidence

The transport of water vapor from wetted filter paper discs in a vapor pressure psychrometer chamber was used to assess experimentally the time required for vapor equilibration. It was assumed that the filter paper offered little resistance to the movement of water vapor from the surface to the chamber atmosphere. Under these conditions, vapor transport should occur quickly according to calculations presented by Lambert and van Schilfgaarde (1965). Rapid vapor equilibration in the psychrometer chamber would allow one to track the

change in the total water potential with time in the soil [illegible] growing tissue.

Materials and Methods

An experiment was conducted to determine the time constant for vapor equilibration in a psychrometer chamber containing wetted filter paper discs. Into each of thirty-six psychrometer chambers were placed one 9 mm diameter filter paper disc which had been soaked in deionized water. The chambers were initially at 25°C, and the chamber relative humidity was assumed to be identical to that of the room (65%). Chambers containing filter paper discs were attached to vapor pressure psychrometers and immersed in a constant temperature (30°C) water bath. Water potentials, which reflected the degree of vapor saturation in the chambers, were recorded at least every 7 min. over a 6 h period for each psychrometer with a dew point microvoltmeter.

Results

Complete vapor equilibration was deemed to have occurred when output from each psychrometer was less than 0.3 uv. This point was attained in 67 ± 11 minutes which indicates that without a barrier to diffusion, a considerably longer period of time was required to saturate the psychrometer chamber than was estimated by eqn. A-8 and boundary conditions given by the equations A-9 and A-10.

[illegible] Thermodynamic properties of air, water and brass system components and the interfacial areas of the water bath psychrometer tissue system

Variable name symbol units	Air	Water	Brass wall
Heat capacity Cp (J kg^{-1} K^{-1})	10.0057 X 10^{2}	4.1818 X 10^{3}	0.385 X 10^{3}
Coef. thermal expansion, B (°K^{-1})	3.30 X 10^{3}	0.18 X 10^{-3}	
Kinematic viscosity, v (cm^{2} s^{-1})	15.68 X 10^{6}	1.006 X 10^{-6}	
Thermal conduct., K (W m^{-1} K^{1})	2.62 X 10^{2}	5.97 X 10^{1}	
Prandtl #, Pr	7.08 X 10^{1}	7.03	
Thermal diffusiv., a (m^{2} s^{-1})	2.216 X 10^{-5}	1.43 X 10^{7}	3.412 X 10
Mass, (kg)			0.04
Velocity of water of bath (m sec^{1})		0.05	
Density, ρ (g cm^{-3})	0.00165		
Temp. diff., T (°C) 5.0			

Interface	Interfacial areas (mm^{2})
Water bath-chamber walls	740
Chamber walls-chamber air	609
Air-plant tissue	71
Plant tissue chamber walls	131

```
      DIMENSION VACS(1200),NUM(1200)

      INTEGER N,D,Q,T
      COMMON /ONE/ T
      COMMON /TWO/ 1TOTNUM
      COMMON /THREE/AREA

      OPEN(12,NAME='VACS021.DAT',STATUS='NEW')
      OPEN(11,NAME='CELL021.DAT',STATUS='NEW')
C
C INITIALIZE VARIABLES
      STRTVAL=315
      DEL=5.0
      AREA=2.0E-04
C
C FILL ARRAY NUM WITH INITIAL POPN OF CELLS
C
      DO 100 I=1,1200
      NUM(I)=0
      IF (I .GT. 71 .AND. I .LT. 87) NUM(I)=1000000
100   CONTINUE
C
C FILL ARRAY VACS WITH MEAN VALUES FOR THE VOLUME
C AVERAGED CLASS SIZE
C
      VACS(1)=STRTVAL
      DO 200 I=2,1200
      VACS(I)=VACS(I-1) + DEL
200   CONTINUE
C
C
C **********************************************************
C
C BEGINNING TIME INCREMENT OF 1 HOUR
C
      DO 10000 T=1,556
C
C
C THE FOLLOWING SUBROUTINE EXPANDS ALL CELLS
C
      CALL VOL(VACS,NUM)
C
```

```
      IF(ITOTNUM .GT. 6208) GOTO 310
C
C THE FOLLOWING SUBROUTINE DIVIDES A CLA
C OF CELLS
      CALL DIVIDE(VACS,NUM)
C
C WANT TO FIND VOLUME OF EACH CLASS BY MULTIPLYING
C THE NUMBER OF CELLS IN EACH CLASS BY ITS AVERAGE VOLUME.
C
315   ITOTNUM=0
      DO 325 L=1,1200
      ITOTNUM=ITOTNUM + NUM(L)
325   CONTINUE
C
350   TOTV=0.
      DO 400 I=1,1200
      TOTV=TOTV + NUM(I)*VACS(I)
400   CONTINUE
C
      AVEVOL=TOTV/ITOTNUM
C
      TOTV=TOTV*1E 12
C ABOVE LINE COVERTS CUBIC MICRONS TO CUBIC CENTIMETERS
C NEXT LINE ADDS 20% MORE VOLUME TO ACCOUNT FOR AIR SPACES
C NOTE THAT IT IS TIME AND VOLUME INDEPENDENT (1ST CRACK)
      TOTV=TOTV*1.2
C NEXT LINE CONVERTS VOL TO APPROX AREA
      AREA=TOTV/ 015
      WRITE(11,*) T,AREA,ITOTNUM,AVEVOL
C     WRITE(6,*) T,AREA,ITOTNUM,AVEVOL
C
10000 CONTINUE
      STOP
      END
C
C ***********************************************
C
      SUBROUTINE VOL(VACS,NUM)
      DIMENSION P(556),VACS(1200),NUM(1200)
      INTEGER T,Q,E,D
      COMMON /ONE/T
      COMMON /THREE/AREA
C PHE IS THE WALL EXTENSIBILITY
      PI=3 1415
      PHE=0 06
C
      P(T)=3*(1.9 + SIN((T+4)*PI/12))
      Y=( 57/275 )*AREA + 3
C
      DO 10000 I=1200,1, 1
      GG=P(T)-Y
      IF (GG  LT  0  )  GG=0.
```

```
95
96
97
98          Z=VACS(I) + VACS(1)*PHE*GG
99        C
100       C FIND NEW VACS WHICH CONTAINS Z
101             DO 2000 Q=1,1200
102             A=VACS(Q) + 2.5
103             C=VACS(Q)   2.5
104             IF (Z .LE. A .AND. Z .GT. C) THEN
105             E=Q
106             GOTO 2010
107             ENDIF
108       2000  CONTINUE
109       C
110       C     MOVE ALL CELLS FROM OLD VACS TO NEW VACS IF
111       C     NECESSARY
112       C IF THE CELLS DONT MOVE OUT OF THEIR PRESENT VOLUME
113       C THEN
114       2010  IF (VACS(E)  EQ  VACS(1) ) GOTO 10000
115       C
116       C ADD CELLS ALREADY IN OLD VACS TO THOSE BEING MOVED
117             IF (VACS(E)  GT  VACS(1) ) THEN
118             NUM(E)=NUM(1) + NUM(E)
119             NUM(1)=0
120             ENDIF
121       10000 CONTINUE
122             RETURN
123             END
124       C
125       C ***************************************************
126       C
127             SUBROUTINE DIVIDE(VACS,NUM)
128             DIMENSION VACS(1200),NUM(1200)
129             INTEGER T,Q,E,D
130             COMMON /ONE/T
131             COMMON /TWO/ITOTNUM
132       C
133       C CALC TOTAL NUMBER OF CELLS THAT FDC REPRESENTS
134       C
135       C WHERE X IS A CONSTANT BASED ON EARLY EXPONENTIAL GROWTH
136       C
137             X=0.0004
138       C
139             ITOTNUM=0
140             DO 100 J=1,1200
141             ITOTNUM=ITOTNUM + NUM(J)
142       100   CONTINUE
143       C
144             FDC=(EXP(X*T)   1.0)/2.0
145       C
146             FNUM=INT(FDC*ITOTNUM)
```

```
C  STARTING WITH THE LARGEST CLASS, SUBTRACT CELLS UNTIL
C PNUM CELLS ARE REMOVED
      DO 1000 K=1200,1, 1
      IF (NUM(K)  EQ  0) GOTO 1000
      IF (PNUM  LT  NUM(K) ) GOTO 200
      IF (PNUM  EQ  NUM(K) ) GOTO 300
      IF (PNUM  GT  NUM(K) ) THEN
C CALCULATE HALF VOL OF VACS(K)  DETERMINE WHICH VACS
C THESE CELLS NOW RESIDE
      VNEW=VACS(K)/2.0
      DO 50 Q=1,1200
      A=VACS(Q) + 2.5
      C=VACS(Q)   2.5
      IF (VNEW  LE  A  AND  VNEW .GT. C ) THEN
      D=Q
      WRITE(12,*) T,VACS(K)
      GOTO  75
      END1F
50    CONTINUE
C
C ADD THE CELLS FROM NUM(K) (WHICH WILL BE DOUBLED) TO
C NUM(D)
75    NUM(D)=NUM(D) + (NUM(K)*2)
C CALCULATE A NEW PNUM AND RESET NUM TO ZERO
      PNUM=PNUM NUM(K)
      NUM(K)=0
      GOTO 1000
      END1F
C
C THIS PART IS USED WHEN PNUM DOESNT REMOVE ALL CELLS FROM
C NUM
200   VNEW=VACS(K)/2.0
      DO 250 Q=1,1200
      A=VACS(Q) + 2.5
      C=VACS(Q) - 2.5
      IF (VNEW  LE  A .AND  VNEW  GT  C) THEN
      D=Q
      WRITE(12,*) T,VACS(K)
      GOTO 275
      END1F
250   CONTINUE
C
275   NUM(D)=NUM(D) + (PNUM*2.0)
      NUM(K)=NUM(K)   PNUM
      PNUM=0
      RETURN
C NEXT PORTION IS USED WHEN PNUM=NUM
C
300   VNEW=VACS(K)/2.0
      DO 350 Q=1,1200
      A=VACS(Q) + 2.5
      C=VACS(Q)   2.5
```

```
199                [illegible] THEN
200                D=Q
201                WRITE(12,*) T,VACS(
202                GOTO 360
203                ENDIF
204          350   CONTINUE
205          C
206          360   NUM(D)=NUM(D) + (NUM(K)*2
207                PNUM=0
208                NUM(K)=0
209                RETURN
210          1000  CONTINUE
211                RETURN
212                END
```

```
      DIMENSION N(25),S(556),P(556)
      REAL*8 V(25)
C
C N(I) IS THE NUMBER OF CELLS IN THE ITH CLASS
C V(I) IS THE VOLUME OF CELLS IN THE ITH CLASS
C P(T) IS THE TURGOR PRESSEURE IN MPA
C T IS THE ITERATION LENGTH EQUAL TO 1 HOUR
C
      INTEGER T
C
C
C INITIALIZE VARIABLES
C PHE IS THE WALL EXTENSIBILITY
C STRTVAL IS THE SMALLEST INITIAL VOLUME CLASS
C DEL IS THE DIFFERENCE IN VOLUME BETWEEN EACH VOL CLASS
C
      PHE=0.06
      STRTVAL=400
      PI=3.1415
      DEL=25.
C
C FILL VOLUME ARRAY WITH VALUES
C
      V(1)=STRTVAL
      DO 100 I=2,25
      V(I)=V(I-1) + DEL
100   CONTINUE
C
C FILL CELL POPN ARRAY WITH NUMBERS
C
      DO 200 I=1,25
      N(I)=600000
200   CONTINUE
C A IS AREA OF THE LEAF IN SQ. CM
      A=1.1
C*****************************************************
      DO 3000 T=1,556
      P(T)= 3*(1.9 + SIN((T+4 )*PI/12 ))
C YIELD THRESHOLD (Y) IS A FUNCTION OF LEAF AREA
      Y=( 57/275.)*A + .3
C
C EXPANSION OF CELLS FOLLOWS DEPENDENCE UPON THE TURGOR
C YIELD THRESHOLD AND THE WALL EXTENSIBILITY
C
C EACH CLASS OF CELLS IS EXPANDED
```

```
      DO
      GG=
      IF (G
      V(I)=V(I) + V(I)*PHE*GG
       IF (V(I)  GE. 8400 ) V(I
  400 CONTINUE
C
C S(T) IS THE FUNCTION WHICH DESCR
C MUST OBTAIN BEFORE IT MAY DIVIDE
C
      S(T)=12.*T + 900.
C
      DO 400 I=1,25
      IF (V(I)  GE  S(T)) THEN
C
C IF THE VOLUME OF THE CELL IS GREATER
C VOLUME IS HALVED AND IT IS DOUBLED
C
      N(I)=N(I)*2
      V(I)=V(I)/2.0
      ENDIF
  400 CONTINUE
C
C SUM ALL THE CELLS IN EACH CLASS
C
      ITOTNUM=0
      DO 450 I=1,25
      ITOTNUM=ITOTNUM + N(I)
  450 CONTINUE
C
C FIND THE VOLUME OF EACH CLASS BY MULTIPLYING
C THE NUMBER IN EACH CLASS BY ITS VOLUME AND THEN
C SUMMING UP OVER ALL CLASSES
C
      TOTV=0
      DO 500 I=1,25
      TOTV=TOTV + V(I)*N(I)
  500 CONTINUE
C
C
C AVEVOL IS THE AVERAGE CELL VOLUME IN CUBIC MICROMETERS
C
      AVEVOL=TOTV/ITOTNUM
C CONVERT CUBIC MICROMETERS TO CUBIC CENTIMETERS
      TOTV=TOTV*1E-12
C CONVERT SOLID PACK LEAF TO ONE WITH 20% AIR SPACE
      TOTV=TOTV*1.2
C CONVERT LEAF VOLUME TO AREA
      A=TOTV/.015
C
C
```

```
 98          3000  CONTINUE
 99          3500  WRITE(6,*) I, I ,TOTNUM,AVE
100          4000  STOP
101                END
```

REFERENCES

Acevedo, E., T. C. Hsiao, and D. W. Henderson. [illegible]. subsequent growth responses of maize leaves [illegible] status. Plant Physiol. 48:631-636.

Baker, C. K., and J. N. Gallagher. 1983. The development of [illegible] wheat in the field. 2. The control of primordium initiation [illegible] by temperature and photoperiod. J. Agric. Sci. 101:337-344.

Baker, J. T., P. J. Pinter, R. J. Reginato, and S. T. Kanemasu. [illegible]. Effects of temperature on leaf appearance in spring and winter wheat cultivars. Agron. J. 73:605-613.

Barlow, E. W. R., R. Munns, N. Steele Scott, and A. H. Reisner. 1981. Water potential, growth, and polyribosome content of the stressed wheat apex. J. Exp. Bot. 28:909-916.

Bennett, J. M. 1986. Unpublished results. University of Florida, Gainesville, Florida.

Bennett, J. M., and P. M. Cortes. 1985. Errors in measuring water potentials of small samples resulting from water absorption by thermocouple psychrometer chambers. Plant Physiol. 79:184-188.

Berlin, J., J. E. Quisenberry, F. Bailey, and M. Woodworth. 1982. Effect of water stress on cotton leaves. Plant Physiol. 70:238-243.

Boyer, J. S. 1982. Plant productivity and environment. Science 218:443-449.

Boyer, J. S., A. J. Cavalieri, and E. D. Schulze. 1985. Control of the rate of cell enlargement: Excision, wall relaxation, and growth-induced water potentials. Planta 163:527-543.

Boyer, J. S., and E. B. Knipling. 1965. Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. Proc. Natl. Acad. Sci. 54:1044-1051.

Bunce, J. A. 1977. Leaf elongation in relation to leaf water potential in soybean. J. Exp. Bot. 28:156-161.

Carslaw, H. S., and J. C. Jaeger. 1959. Conduction of heat in Solids. Oxford University Press, London.

[illegible]arlss Edwards, D; A 1[illegible] growth. 1979 Ann Bot. 44:523-535.

Cleland, R. E. 1976; The [illegible] cell enlargement. Symp. Soc Exp. Biol 31:101 115

Clough, B. F , and Milthorpe, F. L. 1975 Effects of water deficits on leaf development in tobacco. Aust. J. Plant Physiol 2:291 300.

Cosgrove, D. J. 1981. Analysis of the dynamic and steady state responses of growth rate and turgor pressure to changes in cell parameters. Plant Physiol 68:1439-1446.

Cosgrove, D. J. 1985. Cell wall yield properties of growing tissue Plant Physiol. 78:347-356;

Cosgrove, D. J 1986. Biophysical control of plant cell growth Ann. Rev. Plant Physiol. 37:377-405,

Cosgrove, D. J., and R. E. Cleland. 1983. Osmetic properties of pea stem internodes in relation to growth and auxin action Plant Physiol 72 332-339;

Cosgrove, D. J., E. Van Volkenburgh, and R. E. Cleland. 1984. Stress relaxation of cell walls and the yield threshold for growth. Planta 162 46 54

Curry, R. B., C. H. Baker, and J. G. Streeter. 1975 SOYMOD 1: A dynamic simulator of soybean growth and development. Trans of the ASAE 963 974,

Dale, J. E. 1964 Leaf growth in Phaseolus vulgaris. I. Growth of the first pair of leaves under constant conditions. Ann. Bot; 28:579-589.

Dale, J. E. 1970. Models of cell number increase in developing leaves Ann. Bot 34:267-273.

Dale, J. E. 1976 Cell division in leaves. p. 315-345. In Cell division in higher plants. M. M. Yeomen (ed.) Academic Press, London.

Davies, W. J., and E. van Volkenburgh. 1983. The influence of water deficit on the factors controlling the daily pattern of growth of Phaseolus trifoliates J. Exp. Bot, 34 987 999

Fraisner, R. C. 1920 Daily rhythms of elongation and cell division in certain roots, Amer. J. Bot, 7.380 406;

Gardner, W. R.; and R. H. Nieman. 1964 Lower limit of availability to plants. Science 143 1460 1462.

Green, P. B. 1968. Growth physics in Nitella: A method for continuous in vivo analysis of extensibility based on a micromanometer technique for turgor pressure. Plant Physiol. 43:1169-1184.

Green, P. B., K. Bauer, and W. R. Cummins. 1977. Biophysical model for plant cell growth: Auxin effects. p. 30-45. In Water relations in membrane transport in plants and animals. A. M. Jungreis, T. K. Hodges, and A. Kleinzeller (eds.). Academic Press Inc., New York.

Green, P. B., S. O. Erickson, and J. Buggy. 1971. Metabolic and physical control of cell elongation rate. Plant Physiol. 47:423-430.

Hesketh, J. D., D. L. Myhre, and C. R. Wiley. 1973. Temperature control of time intervals between vegetative and reproductive events in soybeans. Crop Sci. 13:250-254.

Hofstra, G., J. D. Hesketh, and D. L. Myhre. 1977. A plastochron model for soybean leaf and stem growth. Can. J. Plant Sci. 57:167-175.

Horie, T., C. T. de Wit, J. Goudriaan, and J. Benenk. 1979. A formal template for the development of cucumber in its vegetative stage. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C, 82:433-479.

Meise, T. C., and Jing, J. 1984. Biophysical parameters underlying slower expansion of water-stressed maize leaves. Shifts in yield threshold counter to osmotic adjustment. Plant Physiol. 75(1):S174(Abstr.)

Johansen, D. A. 1940. Plant Microtechnique. McGraw Hill Book Co., New York.

Johnson, H. W., H. A. Borthwick, and R. C. Leffel. 1960. Effects of photoperiod and time of planting on rates of development of the soybean in various stages of the life cycle. Bot. Gaz. 122:77-95.

Jones, J. W., and Hesketh, J. D. 1980. Predicting leaf expansion. p. 85-122. In Predicting photosynthesis for ecosystem models. J. D. Hesketh and J. W. Jones (eds.). CRC Press, Boca Raton.

Karlekar, M. V., and R. M. Desmond. 1977. Engineering heat transfer. West Publishing Co. St. Paul, Minnesota.

Kersten, G. 1916. Über embryonales der Kern-und Zellteilungen. Zeitschr. Bot. 10:1-20.

Kaufmann, K. W. 1981. Fitting and using growth curves. Oecologia (Berl.) 49:293-299.

Ballicot A. B. 1904 The daily periodicity of cell division and of elongation in the root of Allium Bull Torrey Club. 31:529 550.

Lambert J. R., J. van Schilfgaarde 1965 A method of determining the water potential of intact plants. Soil Sci. 100:1 9.

Lockhart, J. A. 1965 An analysis of irreversible plant cell elongation J. Theor Biol. 8:264 275.

Loomis, R. S., R. Rabbinge, and E. Ng 1979 Explanatory models in crop physiology. Ann. Rev Plant Physiol. 30 339-367.

Lugg, D. G., and T. R. Sinclair 1981 Seasonal changes in morphology and anatomy of field-grown soybean leaves. Crop Sci. 20:191-196

Makeymowych, R 1973 Analysis of leaf development Cambridge University Press, Cambridge

Masri, M. M. 1986 Leaf expansion and carbon exchange rate of field grown corn and soybean under drought stress. M.S. Thesis, University of Florida, Gainesville, Florida.

Marc, J and J. B. Palmer 1976. Relationship between water potential and leaf and inflorescence initiation in Helianthus annuus. Physiol. Plant 36:101 104.

McCree, K. J., and S. D. Davis. 1974. Effect of water stress and temperature on leaf size and on size and number of epidermal cells in grain sorghum Crop Sci 14:751-755

Meyer, R. F., and J. E. Boyer. 1972. Sensitivity of cell division and cell elongation to low water potentials in soybean hypocotyls. Planta (Berl.) 108 77-87.

Michelena, V. A., and J. S. Boyer. 1982 Complete turgor maintenance at low water potentials in the elongating region of maize leaves. Plant Physiol 69:1145-1149.

Milthorpe, F. L 1956. The relative importance of the different stages of leaf growth in determining the resultant area p 141 151. In The growth of leaves. F. L. Milthorpe (ed.) Butterworths Scientific Publications, London

Milthorpe, F. L 1959. Studies on the expansion of the leaf surface I. The influence of temperature J. Exp. Bot. 10 233-249

Metz, F. J. and J. S. Boyer. 1978 Growth-induced water potentials in plant cells and tissues. Plant Physiol. 62 423-429.

Monteith, J. [illegible]. Climate and the efficiency of crop production in Britain. Phil. Trans. R. Soc. Lond. B. 281:277-294.

Muchow, R., T. R. Sinclair, J. M. Bennett, and L. C. Hammond. 1986. Response of leaf growth, leaf nitrogen and stomatal conductance to water deficits during vegetative growth of field-grown soybeans. Crop Sci. 26:1190-1194.

Peck, A. J. 1969. Theory of the Spanner psychrometer, 2. Sample effects and equilibration. Agric. Meteorol. 6:111-124.

Penning de Vries, F. W. T., and H. H. van Laar. 1982. Simulation of growth processes and the model BACROS. In Simulation of plant growth and crop production. F. W. T. Penning de Vries and H. H. van Laar (eds.). Centre for Agricultural Publishing and Documentation, Wageningen.

Silk, W. K., and K. K. Wagner. 1980. Growth sustaining water potential distributions in the primary corn root. Plant Physiol. 66:859-863.

Sinclair, T. R. 1984. Leaf area development in field grown soybeans. Agron. J. 76:141-146.

Sinclair, T. R., and N. M. Ludlow. 1985. Who taught plants thermodynamics? The unfulfilled potential of plant water potential. Aust. J. Plant Physiol. 12:213-217.

Sinclair, T. R., and N. M. Ludlow. 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. Aust. J. Plant. Physiol. 13:329-341.

Sinclair, T. R., and C. C. Spaeth. 1982. Physiological and environmental limits to soybean yields. In Symposium on physiological aspects of soybean yield. A. Tanaka (ed.). Hokkaido University, Sapporo.

Slatyer, R. O. 1967. Plant water relations. Academic Press, New York.

Sunderland, N. 1960. Cell division and expansion in the growth of the leaf. J. Exp. Bot. 11:68-80.

Sunderland, N., and R. Brown. 1956. Distribution of growth in the apical region of the shoot of Lupinus albus. J. Exp. Bot. 7:127-145.

Takami, S., N. C. Turner, and H. M. Rawson. 1981. Leaf expansion of four sunflower (Helianthus annuus L.) cultivars in relation to water deficits. I. Patterns during plant development. Plant, Cell and Environment 4:359-367.

Terry, N., L J Waldron, and A Ulrich 1971. Effects of [illegible] stress on the multiplication and expansion of cells in leaf [illegible] sugar beet Planta (Berl.) 97:281 289.

Thomas, J F , and C D Raper, Jr. 1976. Photoperiodic control [illegible] seed filling for soybeans Crop Sci. 16:667-672.

Thornley, J. H M , d G Hurd, and A. Pooley. 1981. A model of growth of the fifth leaf of tomato. Ann. Bot. 48:327-340

Thut, H. F., and W E Loomis 1944 Relation of light to growth of plants Plant Physiol. 19:117-130.

Van Volkenburgh, E , and R. E Cleland 1981 Control of light induced bean leaf expansion: Role of osmotic potential, wall yield stress, and hydraulic conductivity Plants 153:572-577.

Van Volkenburgh, E., and R E. Cleland 1986. Wall yield threshold and effective turgor in growing bean leaves Planta 167:37 43.

Vendeland, J. S. 1981. A field evaluation of the plastochron index as a measure of the growth rate of soybeans M S Thesis. Cornell University, Ithaca, New York.

Vandeland, J S., T. d Sinclair, d C Spath, and F M Cortes. 1982: Assumptions of plastochron index Evaluation with soybeans under field drought conditions Ann Bot 50:673-680.

Wenkert, W , E d; Lemon, and T R Sinclair. 1978. Leaf elongation and turgor pressure in field grown soybean Agron. J. 70 761-764.

Wiersma, J. V , and T. E. Bailey. 1975. Estimation of leaflet, trifoliolate, and total leaf areas of soybeans Agron J 67:26 30

Wilson, G. L. 1966. Studies on the expansion of the leaf surface V Cell division and expansion in a developing leaf as influenced by light and upper leaves, J Exp. Bot. 17 440 451.

Yegappan, T M., D M Patton, C T, Gates, and W J. Muller 1980. Water stress in sunflower (Helianthus annuum L.): I. Effect on plant development Ann Bot 46:81 70.

Ziemmermann, U., D. Husken, and E D Schulze. 1980 Direct turgor pressure measurements in individual leaf cells of Tradescantia virginiana: Planta 149:445-453.

Zur, d. and J. W Jenas 1981. A model for the water relations, photosynthesis and expansive growth of crops. Water Resources Res; 17:311:320

[redacted] sburgh, [redacted] graduated from Temple Univers[redacted] 1974, with a B[redacted] (psychology) He joined the Peace Corps in April, 1974, and served [redacted] Senegal, West Africa, until August, 1976 He then entered the University of Florida to obtain training in tropical agricultural sciences in the fall of 1976. He began graduate studies in 1979 and received a Master of Science (horticultural science) with a specialization in vegetable crops from the University of Florida in 1983. He is married and has two perfect children.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. T. R. Sinclair, Chairman
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. G. H. Emerson
Professor of Agricultural Engineering

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. K. J. Boote
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. J. W. Jones
Professor of Agricultural Engineering

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

J. M. Bennett

Dr. J. M. Bennett
Associate Professor of Agronomy

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1986

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School